



PHD

The role of physical activity in maintaining muscle health in healthy older adults

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The role of physical activity in maintaining muscle health in healthy older adults

Oliver Joseph Perkin

A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department for Health

September 2018

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Abstract

Loss of muscle mass and strength are seemingly accepted as part of ageing process, despite ultimately leading to loss of independence. Maintaining a physically active lifestyle is regarded as the primary defence against loss of muscle function in older age. The present thesis aimed to examine the role of physical activity and exercise in maintaining leg muscle function in older adults. Associations between habitual physical activity and leg muscle function were explored in a cross sectional study of healthy older adults. Physical activity *per se* was not associated with leg muscle strength and power, in either absolute terms or relative to muscle size. Thereafter, in a randomised control trial, a homebased exercise strategy employing twice daily short bouts of unloaded exercise was identified as capable of producing measurable increases in leg muscle function in a one minute sit-to-stand test. Trends for increased thigh muscle cross sectional area and leg pressing strength and power, were also recorded. In a second randomised control trial, that ‘exercise snacking’ model was explored as means to prevent the anticipate loss of leg muscle mass and function in healthy older adults undertaking two weeks of reduced physical activity by way of step-reduction to <1,500 steps/day. Contrary to some previous literature, the step-reduction intervention induced losses of leg muscle function, however undertaking concomitant exercise snacking was demonstrated to be a promising countermeasure against reduced activity induced loss of dynamic leg strength and power. In summary, exercise snacking may be sufficient to increase leg muscle size and function in healthy older adults, and provide a feasible means to prevent loss of muscle function during periods of reduced activity.

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Publications

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Perkin OJ, McGuigan MP, Thompson D, Stokes K (2018) Habitual physical activity levels do not predict leg strength and power in healthy, active older adults. *PLoS ONE* 13(7): e0200089.

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‘Daily physical activity characteristics of older versus younger individuals’ British Society for Research on Ageing, Liverpool, 2014

‘Leg strength, leg power, and physical activity, in older versus younger individuals’ American College of Sports Medicine Integrative Physiology, Miami, 2014

‘Movement co-ordination variability in older adults: consistent or constrained?’ Arthritis Research UK (ARUK) Physical Activity and Osteoarthritis, Loughborough, 2015

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Abbreviations

1-RM: 1-repetition maximum force

3MeHis: 3-methylhistidine
microdialysis

4E-BP1: eukaryotic initiation factor
4E binding protein

6MWT: six-minute-walk-test

ANOVA: analysis of variance

AP range: anterior-posterior
displacement range

BM: body mass

BMI: body mass index

BMR: basal metabolic rate

CHO: carbohydrates

CI: confidence interval

CoP: centre of pressure

CRP: C-reactive protein

CSA: cross sectional area

CT: computed tomography

dH₂O: distilled water

DXA: dual energy X-ray
absorptiometry

EAA: essential amino acids

ES: Exercise Snacking

F_{max}: extrapolated maximum
theoretical force

FSR: fractional synthetic rate

GRF: ground reaction force

HD: 6° head down tilt

IL-6: Interleukin-6

ITR: interpolated twitch ratio

MAFbx: Muscle Atrophy F-box

mCSA: muscle cross sectional area

METs: metabolic equivalent of
task compared to the energy cost of
sitting quietly

MG: medial gastrocnemius muscle

MHC: myosin heavy chain

MHz: mega hertz

MPB: muscle protein breakdown

MPS: muscle protein synthesis

MRI: magnetic resonance imaging

mRNA: messenger ribonucleic acids

mTOR: mechanistic target of
rapamycin complex 1

MuRF1: Muscle Ring Finger 1

MVC: maximum voluntary
contraction

MVF: maximal voluntary force

MVPA: moderate to vigorous activity

NHS: National Health Service

NICE: National Institute for Health
and Care Excellence

p70S6K: phospho-70 kDa S6 protein
kinase

PAL: physical activity level as a ratio
of TEE to BMR

P_{max}: interpolated maximum
theoretical power

P_{max40}: mean power of eight reps at
40% 1-RM

pQCT: peripheral quantitative
computed tomography

PRO: proteins

RDA: recommended daily allowance

REC: research ethics committee

RPE: ratings of perceived exertion

SD: standard deviation

SPPB: Short Physical Performance Battery

SR: step-reduction only group

SR+ES: step-reduction with exercise group

STS: 60 second sit-to-stand test

Tc: control twitch

TEE: total energy expenditure

TNF- α : tumour necrosis factor- α

Ts: evoked twitch

UK: United Kingdom

US: ultrasound

USA: United States of America

VL: Vastus lateralis muscle

V_{max}: extrapolated maximum theoretical velocity

Chapter 1: Introduction and review of the literature

1.1 Overall thesis aims and structure

This thesis aims to investigate the role that physical activity plays in maintaining muscle function in healthy older adults by undertaking three experimental investigations. Loss of muscle mass and strength are considered to be an unavoidable and often accepted part of ageing, whilst concurrently older adults typically become progressively less physically active with increasing age. There is a huge range in functional capacity in older adults. The extremes are represented by those with frailty and such limited function that tasks of daily living are impossible, to master's athletes, who exhibit a biological ages decades younger than they are, capable of incredible feats of physical performance, despite being the same chronological age as a frail individuals. The majority of the population of older adults fall towards the middle of this theoretical continuum, and so will likely have the physiological capacity to improve function markedly. This thesis aims to investigate how subtle or short duration changes in physical activity levels can move healthy older adults along this continuum of physical function, with specific attention on strength and power.

The first study sets out to investigate whether there is a relationship between habitual physical activity levels *per se*, and leg strength and power in healthy, generally active older adults. Physical activity is considered in a non-movement specific manner, i.e. the total daily energy expenditure compared to basal energy expenditure, and in terms of total time spent performing movements classified as above the threshold for moderate to vigorous activity, based on metabolic equivalents of movement compared to the energy cost of sitting quietly. Leg strength and power is also compared to younger adults, and considered relative to the thickness of *vastus lateralis* muscle and to body mass. These secondary comparisons address whether loss of muscle size is the cause of strength and power loss in older adults in a rudimentary manner.

The second pilot study investigates whether older adults who do not undertake exercise on a weekly basis can improve muscle strength and power in 28 days by performing a minimal amount of exercise added to their habitual physical activity levels. The exercise programme is termed 'exercise snacking', and asks participants to perform two daily bouts of five minutes of unloaded exercise. The exercises are all

focussed on moving either body weight (sit-to-stand from a chair, standing calf raises) or lower limbs only (march on the spot, seated knee extension, standing knee bend), through as many repetitions as possible in 60 seconds. The only malleable training variable that allows progression is the number of repetitions completed in each minute of each exercise. Alongside leg strength and power, basic tests of ambulatory function and static balance, assessment of neural drive, and measurement of body composition, muscle mass, and muscle cross-sectional area are presented.

The final feasibility study attempts to describe the impact of dramatically reducing daily physical activity levels for two weeks on muscle function in healthy older men, and examine the effects of performing thrice daily exercise snacking during the period of reduced physical activity. A step-reduction model to limit daily walking to less than 1,500 steps is employed in healthy, active older men. Again, neural drive, postural sway during static balance tasks, and scanning measures of muscle size and body composition are used as secondary outcomes.

1.2 General Introduction and review of the literature

1.2.1 Overview

As the UK population continues to grow, it is also getting older, with proportionally more adults living longer (Office for National Statistics 2017). Ageing is associated with decreasing skeletal muscle mass at a rate of around 0.5-1% per year after the age of 50 years, with concomitant loss of muscle force generating capability (Mitchell *et al.* 2012). Furthermore, muscle strength is lost two to five times more rapidly than would be expected based on the rate of muscle mass loss during ageing, and at twice the rate in the legs compared to the upper body (Degens and Korhonen 2012; Seene and Kaasik 2012). Frailty is largely caused by this loss of muscle mass and strength, and is associated with increased risk of falls and reduced quality of life (Faulkner *et al.* 2007; Marcell 2003). Postponement of physical frailty through maintenance or improvement in physical function in older people would have immediate economic, personal and societal benefits. For example, in 1999, the cost of falls in over-60's to UK government was £981 million, with 66% of the costs attributed to falls in adults aged 75 and over (Scuffham *et al.* 2003).

Maintaining an adequate level of physical activity is regarded as crucial for preserving muscle function into later years of life (Manini and Pahor 2009), and it has long been recognised that even short term periods of disuse causes muscles to lose both size and strength (Creditor 1993). This has been demonstrated experimentally with a variety of disuse models in humans, including bed-rest, and limb immobilisation by bracing or casting, or unilateral limb suspension (Hackney and Ploutz-Snyder 2012; Trappe *et al.* 2004). However, reasonably recent evidence from Breen *et al.* (2013) has highlighted that even short term reductions in physical activity by limiting walking to <1,500 steps a day may cause substantial loss of lower limb mass. Irrespective of the form of unloading employed, older individuals seem to be more vulnerable to disuse-induced muscle atrophy than younger individuals (Urso *et al.* 2006; Wall *et al.* 2013a).

Episodes of dramatically reduced physical activity become more common into older age, and may contribute to long term loss of muscle mass, and likely function (Gianoudis *et al.* 2015; Marcell 2003). As portrayed in Figure 1.1, whilst the progressive loss of muscle is considered linear at a population level, at an individual

level it is likely to be responsive to ‘catabolic crises’ (English and Paddon-Jones 2010). Periods of physical inactivity following everyday occurrences such as illness or extended periods of inclement weather may represent catabolic crises. In the window of time following losses of muscle mass and strength due to reduced activity, individuals may have reduced function, making tasks of independent daily living relatively more challenging, and are potentially at greater risk of falls. Finding safe and cost effective strategies to counteract step-reduction induced loss of muscle mass may be an effective means to delay sarcopenia and frailty.

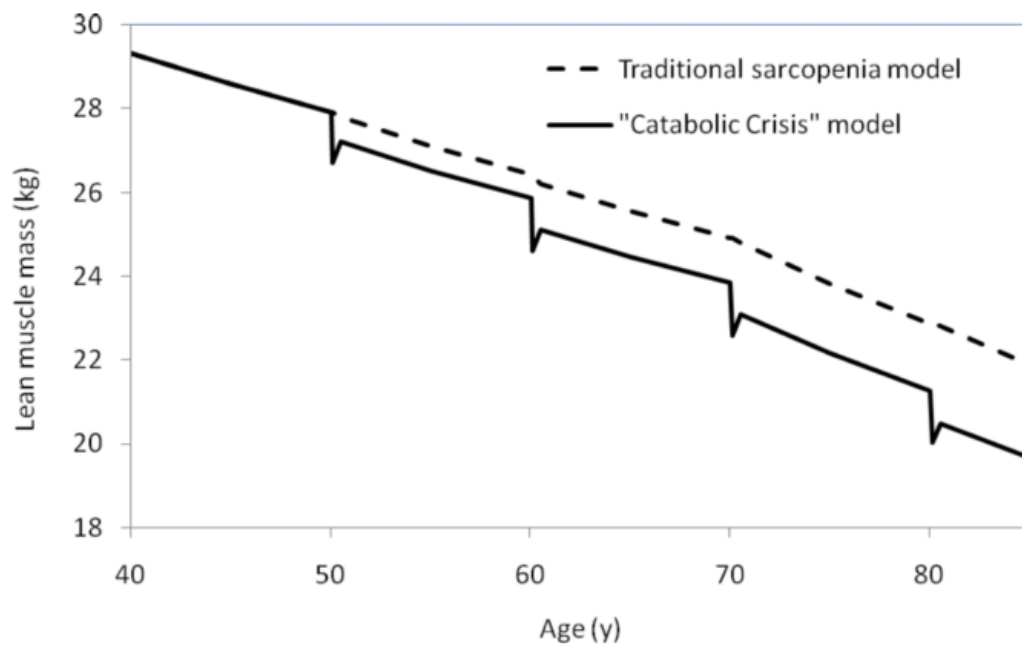


Figure 1.1. Taken from English and Paddon-Jones (2010), a schematic visualising the proposed pattern of muscle loss in an ageing individual, characterised by acute drops in lean muscle mass due to ‘catabolic crisis’.

1.2.2 The ageing population

In July 2017, the Office for National Statistics estimated the UK population to be 65 million people, of which 18% were aged 65 years or over. In the next 10 years, it is projected that the population will rise to ≈ 70 million with 20% being 65 years or over, and by 2045, a quarter of the projected population of ≈ 76 million people will be over 65 years or over (Office for National Statistics 2017). Ageing is associated with increased prevalence of many long term diseases and health conditions, of which musculoskeletal disorders rank high in terms of personal cost to the individual, and economic cost to society (Boirie 2009; Booth and Zwetsloot 2010; Fried *et al.* 2001). The NICE Clinical Guidelines (2013) on Falls; Assessment and prevention of falls in older people, estimated that the cost of falls in adults aged >65 years to the NHS alone to be £2.3 billion. Whilst this economic cost is striking, the personal impact of reduced physical function and subsequent loss of independence, and the associated cost of social care should not be overlooked.

1.2.3 Ageing muscle

The importance of maintaining adequate skeletal muscle mass and strength throughout the lifespan is well recognised. Ageing is associated with decreasing muscle mass (sarcopenia), and evidence is unequivocal that periods of unloading of skeletal muscle also cause reductions in muscle cross-sectional area (mCSA), volume, mass, and strength in both young and older populations (Wall *et al.* 2013a; Kortebein *et al.* 2007; Bodine 2013; Deschenes *et al.* 2008; Hvid *et al.* 2010; Suetta *et al.* 2009; Coker *et al.* 2015; Rejc *et al.* 2015). The annual rate of muscle mass loss after the age of 50 years is estimated to be around 0.5-1 % (Mitchell *et al.* 2012), although this figure is subject to large variation between individuals, due to a combination of genetic and lifestyle factors, and between muscle groups within individuals, with lower limb muscles typically exhibiting greater rate of atrophy with age than upper limb muscles (Degens and Korhonen 2012). This may be primarily down to a greater relative reduction in use of the muscles of the legs compared to the arms into older age (Degens and Korhonen 2012). Whilst a clear relationship exists between mCSA and force generating capacity in healthy, young individuals, it is apparent that the rate of strength loss with age is accelerated compared to mCSA loss (Seene and Kaasik 2012). Loss of isokinetic knee extensor torque occurs at 2-5 times the rate that would be expected

based on muscle mass loss (Delmonico *et al.* 2009). This mismatch between muscle size and force producing capability with ageing is likely to be due to progressive spinal motor unit loss, with incomplete reinnervation of denervated muscle fibres, along with reduced motor unit firing capacity (Aagaard *et al.* 2010). With this shift in motor unit number, it appears that there is also a shift towards proportionally greater numbers of type I fibres per muscle, and a less uniform distribution of fibres throughout the muscle, also contributing to lower force producing capability (Andersen 2003).

The loss of muscle strength with age is commonly termed dynapenia, and is regarded as a primary cause of the loss of functional independence in older adults. Indeed, frailty is largely caused by this loss of muscle function (Faulkner *et al.* 2007; Marcell 2003). Eventually, if strength loss is unchecked, tasks of daily living eventually become too physically strenuous to be managed safely, and independent living simply becomes untenable below this ‘frailty threshold’ (Fried *et al.* 2001). As ageing associated loss of strength and function progresses, the risk that individuals might cross this theoretical frailty threshold due to injury or ‘catabolic crisis’ increases, a concept illustrated in Figure 1.2 (Cesari *et al.* 2016; English and Paddon-Jones 2010). Furthermore, decline in muscle mass in any population is generally allied with negative changes to body composition, i.e. accrual of fat mass (both in absolute terms and relative to lean tissue), and the associated deterioration of metabolic health and elevation in markers of systemic inflammation derived from adipose tissue (Patel *et al.* 2011; Cesari *et al.* 2005). Both ageing and physical inactivity are also independently associated with increased systemic inflammation and oxidative stress, with circulating inflammatory cytokines (tumour necrosis factor- α (TNF- α), Interleukin-6 (IL-6), C-reactive protein (CRP)) implicated in further potentiation of muscle wasting (Visser *et al.* 2002; Schaap *et al.* 2006; Cesari *et al.* 2005).

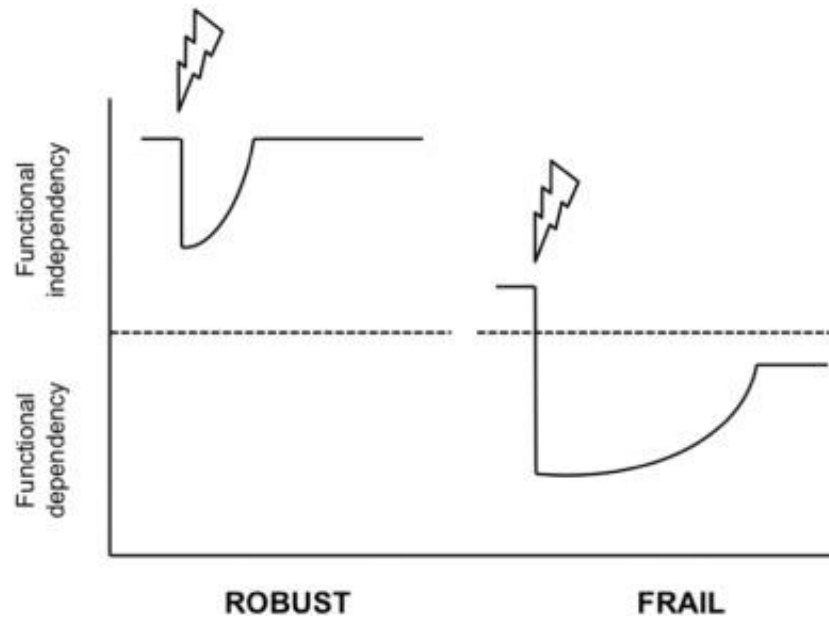


Figure 1.2. A schematic taken from (Cesari *et al.* 2016) to highlight that as individuals becoming increasing close to a threshold of function, below which they are no longer capable of independently carrying out tasks of daily living, a ‘catabolic crisis’ (as depicted by the lightning bolt) is more likely to render them functionally dependant.

1.2.4 Muscle function and physical activity

Sarcopenia is defined as the loss of skeletal muscle mass with age, and the physiological implications of reduced muscle mass have been widely researched (Boirie 2009). Muscle mass and force producing capability are of course inherently linked, however into older age the relationship between muscle mass and strength becomes less clear (Reed *et al.* 1991). Moreover, from a functional perspective it is the loss of maximal strength with ageing that is in fact the cause of loss of function, not muscle mass *per se* (Clark and Manini 2008). Indeed, health related outcomes in older adults are better predicted from measures of strength than they are from muscle mass. Menant *et al.* (2016) compared four traditional body mass based definitions of sarcopenia using dual energy X-ray absorptiometry (DXA), to seated knee extensor strength assessment, for predicting balance and falls related health outcomes in older adults. It was found that strength was a better index for predicting performance in functional tests, and falls over one year of follow up. Moreover, the percentage of participants classified as sarcopenic using the body mass based measures ranged from 14 to 73% depending on which definition was used. Beyond functional implications of strength in older adults, as part of the Health, Aging and Body Composition Study, Newman *et al.* (2006) demonstrated that isokinetic knee extensor strength strongly predicted mortality, but muscle quantity measured by DXA and computed tomography (CT) did not. Of the 2292 non-disabled participants screened at baseline, 286 died over the 5 year follow up, and the unadjusted hazard ratio for knee extensor strength was 1.54 (95% confidence interval (CI); 1.32-1.79) per standard deviation (SD) of 38 Nm. This clearly demonstrates the importance of muscle strength in older adults. In simplistic terms, the stronger that an individual is, the easier and therefore safer tasks of daily living are. Moreover, with more strength comes greater capacity to complete tasks of daily living, allowing older adults to stay more active into later life, so delaying the loss of functional independence (Marcell 2003).

Strength is relatively easy to measure in older adults, with isokinetic knee extensor strength commonly quantified in literature on healthy ageing (Goodpaster *et al.* 2006). However, in day-to-day living, voluntary movements requiring relatively high force production are likely to require rapid execution, such as in trip recovery (Pijnappels *et al.* 2008). Muscle power is the product of force and velocity of movement, and it is evident that power decreases with age to an even greater extent

than strength (Lanza *et al.* 2003). Power is considered to be a better predictor of functional status than muscle mass or isometric or isokinetic strength alone (Cooper *et al.* 2013; Bean *et al.* 2002). Frontera and Ochala (2015) suggest that maximum power of a muscle is generated at around 40% of maximum force, and 15-20% of maximum velocity, although this would be variable in multi-joint movement driven by multiple muscle groups. Accurate and safe measurement of peak power in older adults is challenging due to safety and technique related issues, however pneumatic resistance exercise machines, when instrumented to record kinetic data, are accurate, valid, and reliable, in the assessment of lower limb muscle power, and allow extrapolation or interpolation of peak force, velocity, and power (Reid and Fielding 2012; Beaudart *et al.* 2015; Bobbert 2012).

It is important to define that the characteristics of whole limb movement capabilities cannot be taken as direct representation of the characteristics of the muscle tissue itself. The characteristics of whole limb movement will be inherently impacted by the complex coordination of muscle groups, the morphological properties of those muscles (fibre pennation angle, size, fat infiltration etc.), along with the compliance of the non-contractile tissues of the muscle. Moreover, these additional structures and components of a limb make it impossible to precisely determine force that the muscle itself is producing in isolation at any time, and as such preclude the possibility to measure true isotonic contraction *in vivo*. This is important when considering methodology used to describe changes in muscle characteristics, with isolated and chemically skinned single muscle fibres evidently behaving differently to muscles within whole limbs of living organisms. For example, at the single fibre level contractile properties of muscle of healthy men aged over 70 years are protected to some degree by physical activity (walking at least two hours a day) compared to being sedentary (D'Antona *et al.* 2007). At the whole body level, the relationship between habitual physical activity and muscle power or strength in healthy older adults is surprisingly unclear. Some studies demonstrate associations between leg power and daily moderate intensity physical activity level (Straight *et al.* 2016). In mobility-limited older men, leg press strength was weakly correlated with physical activity counts from a waist mounted accelerometer, but leg press power was not (Morie *et al.* 2010). Similarly, no association was found between changes in isokinetic knee extensor strength and physical activity over 10 years in a wide range of physically

functioning older men and women (aged ~70 years at follow-up) (Hughes *et al.* 2001). The authors suggested that this cohort may have been above a threshold at which the habitual physical activity levels assessed by questionnaire would have a direct effect on muscle strength. However, in 96, generally healthy, community dwelling women aged 74 (6) (mean (SD)) years, questionnaire reported daily moderate intensity physical activity levels were associated with lower limb power independent of lower limb muscle mass or resistance training (Straight *et al.* 2016). Overall, current understanding of the relationship between habitual physical activity levels and lower limb muscle power in active, healthy older individuals is incomplete and often limited by the self-report techniques that have been used to date.

1.3 The impact of short term physical inactivity

Maintenance of muscle mass is the result of continual steady turnover of muscle protein. Research exploring mechanisms for skeletal muscle loss has been centred on the basic concept that the balance of concurrent muscle protein synthesis (MPS) and muscle protein breakdown (MPB) shifts towards a state of net muscle protein loss (Gibson *et al.* 1987), with comprehensive reviews of the mechanisms available (Burd *et al.* 2012; Rennie *et al.* 2004; Phillips *et al.* 2009; Hackney and Ploutz-Snyder 2012). To clarify the minutiae of this negative shift in muscle protein turnover brought about by muscle disuse in humans, previous studies have used extreme interventions to unload participants' muscles such as prolonged periods of bed rest or unweighting of a limb, producing stark physiological changes that have been readily quantifiable (Coker and Wolfe 2012; Rittweger *et al.* 2005; Trappe *et al.* 2008; Trappe *et al.* 2004). These complete models of unloading have provided a wealth of knowledge on the physiological processes at play during muscle wasting in various populations and muscle groups. However, total unloading in real world scenarios are likely to be accompanied by severe challenges to physiological homeostasis such as disease and injury, with these complete disuse models often providing a surrogate for zero gravity space flight, which bears little applicability to older individuals. Furthermore, in older individuals severe health challenges resulting in bed-rest are commonly associated with protein under-nutrition, itself exacerbating muscle loss, which is ethically challenging to include in clinical studies (Sullivan *et al.* 1999; Tieland *et al.* 2012). Common scenarios faced by healthy older individuals in everyday life that lead to reduced physical activity may include sustained inclement weather, suffering a minor injury or illness, or undergoing elective surgery which do not result in extended total unloading (Cohen-Mansfield *et al.* 2003; Grossman and Stewart 2003).

1.3.1 Total unloading models

Traditionally research exploring the effect of unloading of muscles using human participants has tended to implement models of entire lower body muscle disuse through bed-rest, or unilateral limb disuse via immobilisation by casting, bracing, or suspension. Total unloading interventions, in particular bed rest with a 6° head-down tilt (HD), have commonly been used to mimic the effects of zero gravity space flight on muscle and bone turnover (Fitts *et al.* 2010). These studies have consistently demonstrated that total unloading of muscle induces substantial declines in muscle mass and strength. For example, young, healthy male participants undertaking HD bed rest for 90 days experienced a 26 (3) % decline in calf mCSA measured using peripheral quantitative computed tomography (pQCT) (Rittweger *et al.* 2005). In fact, even participants performing 28 maximal concentric and eccentric supine squats and calf press every third day for the duration of bed rest still lost 17 (3) % of calf mCSA. Using magnetic resonance imaging (MRI) Trappe *et al.* (2004) reported a 17% loss of quadriceps muscle volume from baseline in a group of 6 healthy males following 84 days of HD bed rest when provided no protective intervention. Of note, maximum voluntary contraction (MVC) during isometric squats was reduced by 43% in this group, and peak power by 47%.

Long term and complete unloading models are generally designed with the sole focus of understanding the microgravity effects on astronauts, so there is an inherent need for the duration of unloading interventions to match potential lengths of space flight. However, even relatively short duration disuse has been shown to induce marked reductions in muscle size, and are more relevant to hospital stay related muscle disuse, albeit in the absence of any clinical complications. A study by Suetta *et al.* (2009) observed quadriceps muscle volume decreases of 8.9% in young males subjected to 14 days of limb immobilisation through casting, with the knee set at 30° flexion to prevent any load bearing. These findings are comparable with the 5 (1) % decline in quadriceps mCSA reported by Glover *et al.* (2008) using a similar two week immobilisation intervention, which also induced a 25 (3) % drop in peak isometric torque. In young males, five days of HD bed rest resulted in 3% reductions in both thigh and calf mCSA, even when participants were allowed to stand for 25 minutes a day (Mulder *et al.* 2015). Furthermore, without the daily standing during bed rest, knee

extensor isometric strength was decreased by 8%, although there was no loss of plantar flexion strength.

A study by Miokovic *et al.* (2012) examining muscle volume from MRI of the whole lower limb pre-, mid-way through, and post- 60-day HD bed rest highlighted the non-uniformity in mCSA loss between individual muscles. All muscles of the lower limb were measured in this study, with the most pronounced atrophy occurring in the *gastrocnemius medialis* and *soleus*, and then *vasti* muscles of the thigh, followed by *tibialis anterior*, the hamstrings, and *adductor magnus*. Furthermore, atrophy was not uniform across the muscle length in 12 of the 19 muscles measured, and the muscle sub-region of greatest atrophy did not necessarily correlate with the point of greatest mCSA. Uniform atrophy only occurred in the *soleus*, *adductor brevis*, *gracilis*, *pectineus*, and *extensor digitorum longus* muscles. The authors suggest that reasons for differential atrophy between muscles and also across muscle lengths are both likely to be related to the specific use of the individual muscles and sub-regions of those muscles in everyday life, with the muscles and sub-regions used most often suffering the greatest disuse induced atrophy. Anatomical differences between muscles also likely plays a part in explaining inter-muscle differences in atrophy rate, however it was noted that information regarding functional or anatomical compartmentalisation for a number of lower limb muscles was not present in the literature for humans. This information may be important when examining loss of muscle force generating capability across a range of joint angles after a period of unloading inducing muscle loss.

1.3.2 Ageing exaggerates disuse induced muscle atrophy with total unloading

Older individuals show an amplified susceptibility to lose muscle mass through total lower limb disuse compared to younger individuals (Degens and Korhonen 2012; Wall *et al.* 2013a; Urso *et al.* 2006; Tanner *et al.* 2015). As such, due to the ethical implications of intentionally inducing muscle wasting the participants of previous total unloading studies have tended to be healthy young individuals, especially in studies implementing bed rest. However, in participants aged 68 (5) years, 10 days of bed rest has been demonstrated to induce reductions in DXA measured leg lean tissue mass of 7% (Kortebein *et al.* 2006). Similarly, 10 days of bed rest without protective pharmacological intervention, whole body lean mass was reduced by 2.05 (0.66) kg in older adults (67 (2) years), of which 1.01 (0.35) kg was from the lower extremities (Deutz *et al.* 2013). This rapid loss of muscle mass in older individuals is consistent with findings from a seven day bed rest study involving six 60-73 year olds, observing losses of 3.0% of total lean mass, and 4.1% of lean leg mass (Drummond *et al.* 2012). Despite the duration of bed-rest in these studies with older participants being less than half of 28-day bed rest model used by Paddon-Jones *et al.* (2004), in absolute terms these losses are twice that observed in participants aged 38 (6) years, with the younger participants from the aforementioned study losing less than 0.4 kg with bedrest.

Loss of muscle function also seems to be exaggerated in older individuals compared to younger individuals in response to complete unloading (Trappe 2009). Both isometric muscle strength relative to muscle volume and the rate of force development decreased significantly more in the older than younger men following two weeks of unilateral leg casting (Hvid *et al.* 2010). Surprisingly, older individuals lost relatively less individual muscle fibre size, and only showed significant cross sectional area (CSA) loss of type IIa fibres whereas younger individuals lost CSA in all three fibre types. This aligns with the findings of Nilwik *et al.* (2013) that reduced type II muscle fibre size is the main cause of skeletal muscle mass loss with ageing. Type II fibres are generally associated with explosive movements, but Hvid *et al.* (2010) did not identify any significant associations between loss of type II fibre area and rate of force development capacity after the immobilisation period in either age group. This likely implicates neural changes in the loss of muscle function during limb unloading, especially in the older group. Previously reported data from the same investigation demonstrated that older individuals displayed reduced neuromuscular

activation capabilities during maximal voluntary contraction, which the younger group did not (Suetta *et al.* 2009).

1.3.3 Recovery from total unloading models, and the impact of ageing

Recovery of lost muscle mass due to unloading is likely to be more challenging in older individuals compared to younger individuals. Kumar *et al.* (2012) demonstrated that older men required an increased volume of resistance exercise to match the MPS rates achieved by younger men. This supports findings in older women, whereby 12 weeks of resistance training, three times per week, induced 6.2% increases in quadriceps muscle volume in younger women compared to only 2.5% increases in older women (Greig *et al.* 2011). However, marked gains in both muscle size and strength can be achieved in older individuals undertaking resistance exercise regimes. For instance, in a study by Leenders *et al.* (2013) quadriceps mCSA increased by 8% in women and 7% in men after 12 weeks of resistance exercise, with no increase following a further 12 weeks of training. At 12 weeks, strength measured by leg extension 1-repetition maximum force (1-RM) increased by 22% in females and 23% in males, and by 24 weeks had further increased by 17% in females and 16% in males, despite no further increase in quadriceps muscle size. Also, older females completing a 12-week whole body progressive resistance training programme using machine weights increased *rectus femoris* muscle volume by 26% measured using ultrasound (Correa *et al.* 2013). Reid *et al.* (2015) demonstrated increases of 34% and 13% in leg muscle peak power and strength in mobility limited older adults undertaking low load power training twice a week for 16 weeks. This increased muscle function was in part attributed to increased neural activity alongside changes in muscle size, and likely contributed to the improved Short Physical Performance Battery (SPPB) scores observed.

Resistance exercise regimes undertaken specifically to recover muscle size and strength after disuse induced atrophy in an older population have received notably less attention in the literature. A study employing four weeks of retraining after two weeks of unilateral limb immobilisation in older and younger individuals suggests that recovery of muscle size and some aspects of strength may be impaired in older individuals (Suetta *et al.* 2009; Hvid *et al.* 2010). Specifically, Suetta *et al.* (2009) observed that the retraining period did not restore older individuals' quadriceps muscle

volume to pre-immobilisation values, whilst the younger group fully recovered lost muscle volume. Furthermore, whilst isometric force per cm² of mCSA and isokinetic force per kg of body mass were recovered to baseline values in both groups, not all elements of muscle function were fully restored in the older participants, in particular the ability to generate force during the initial phase of muscle contraction (0-50 ms) remained impaired (Hvid *et al.* 2010). However, a more recent study by Hvid *et al.* (2014) suggests the time course of muscle strength recovery may require more scrutiny. Following four days of leg immobilisation, seven days of recovery did not restore isometric or isokinetic knee extensor strength in older individuals, whilst younger individuals' strength returned to baseline. The retraining regime used in the three studies mentioned implemented unilateral lower limb resistance exercise. Although this model was shown to induce measurable gains in muscle size and strength in older individuals post hip replacement surgery (Suetta *et al.* 2004), the total training volume, and probably therefore training responses, were not as great as those seen in the whole body resistance training studies of older adults such as those by Leenders *et al.* (2013) and Correa *et al.* (2013). There is also no mention of control for dietary protein intake during the retraining periods in these studies, which may be an important factor in maximising the anabolic efficacy of resistance exercise in older individuals (Wall *et al.* 2014a).

An additional and critically important role of the neuromuscular system is to maintain postural balance. If the neuromuscular system is compromised after a period of reduced activity it could be hypothesised that balance would be affected leading to an increased risk of falling (Butler *et al.* 2008; Cook *et al.* 2014). As mentioned previously, fall risk is a primary concern in older adults, and there is evidence that balance may be affected by disuse. In young males, it has been demonstrated that postural sway is increased significantly after a 20-day HD bed rest (Kouzaki *et al.* 2007). This compromised balance when standing was evident even in a group that had undergone leg press and calf extension exercise throughout bed rest, and maintained plantar flexor muscle volume. It has long been recognised that postural sway is related to fall risk in older adults, albeit not as strongly as might be anticipated (Ferne *et al.* 1982). Nonetheless, increasing strength through resistance exercise after a period of unloading may have the secondary benefit of restoring losses in balance (Pijnappels *et al.* 2008).

Consideration should be given to the fact that if recovery of muscle size was dramatically impaired in older individuals, then the long term consequence of disuse induced atrophy would likely result in a much more severe annual loss of lean tissue than the 0.5-1% reported in the population data (Mitchell *et al.* 2012). It may be that older individuals need a longer or more intensive retraining period to recover from disuse induced atrophy compared to younger individuals, with the need for adequate protein consumption becoming crucial with advancing age (Suetta *et al.* 2009; Hvid *et al.* 2014; Greig *et al.* 2011). Nonetheless, it is clear that disuse induced atrophy poses a physiological challenge to older individuals; a concept illustrated in Figure 1.3.

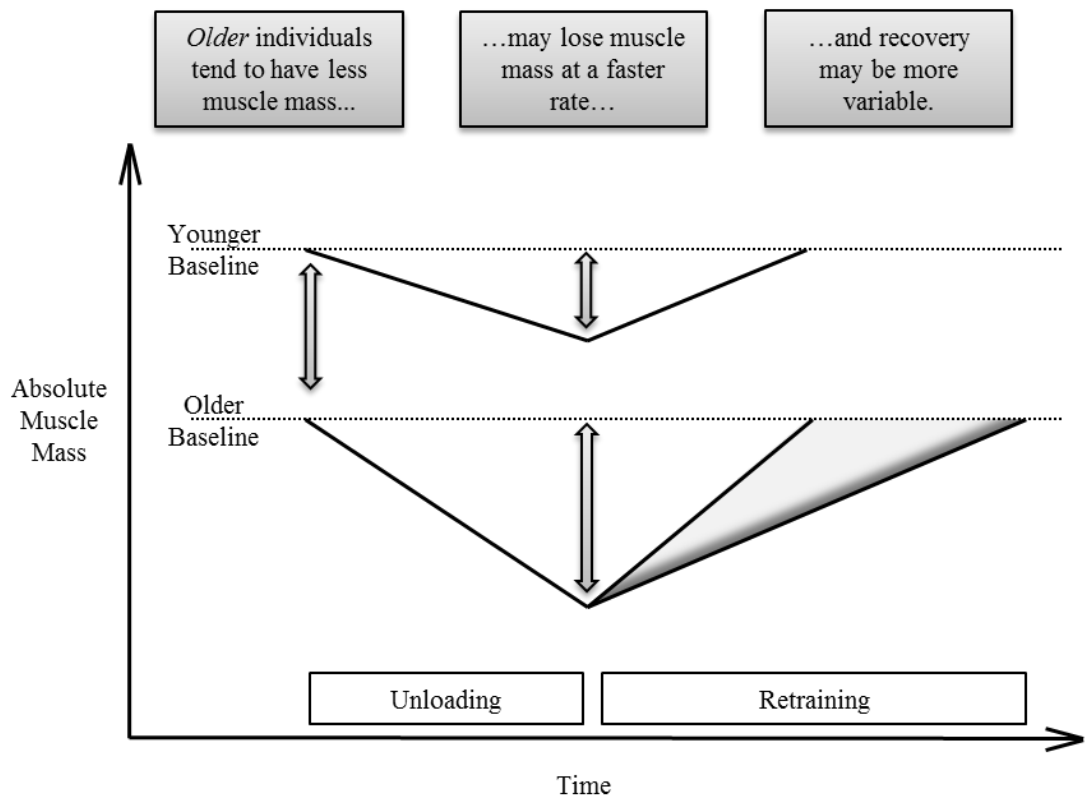


Figure 1.3. Schematic of the differences in muscle mass changes in older compared to younger individuals in response to match unloading and retraining protocols (previously published in (Perkin *et al.* 2015)).

1.3.4 A step-reduction model of reduced activity

Dramatic models of muscle unloading have allowed vital examination of the mechanisms resulting in muscle wasting, but these models are not necessarily an accurate reflection of the nature of unloading experienced by the majority of healthy, free-living individuals. Step-defined levels of activity have provided a useful index against which to correlate data on recognised markers of health (Tudor-Locke *et al.* 2013), and as such, a step-reduction model may be a feasible means of implementing controlled periods of reduced activity in experimental studies and an intervention comparable to real world scenarios of reduced activity. This step-reduction model has previously been implemented to explore the impact of limited ambulation on metabolic changes (Dixon *et al.* 2013; Knudsen *et al.* 2012; Olsen *et al.* 2008; Walhin *et al.* 2013). Reducing steps from $\approx 10,000$ to $\approx 1,500$ per day for two weeks has been observed to significantly impair insulin sensitivity, attenuate postprandial lipid metabolism, and increase central adiposity (Thyfault and Krogh-Madsen 2011). These negative implications are widely considered to play major roles in the deterioration of metabolic health leading to chronic disorders such as Type 2 diabetes (Booth and Hargreaves 2011). A few of these studies have included measures of muscle mass changes from pre-to-post-step-reduction which have provided the initial indication that measurable changes in leg muscle mass may be observed with as little as two weeks of reducing daily ambulation to less than 1,500 steps (Table 1.1).

Table 1.1. Studies using step-reduction models with assessment of changes in muscle mass. (*Continued overleaf*)

Reference	Subjects	Step-reduction (Duration and steps/day)	Measures of muscle mass reported	Results
(Breen <i>et al.</i> 2013)	10 older adults; 5 women (72 (1) years)	14 days 5,962 (695) to 1,413 (110)	DXA; whole-body FFM, ALM, leg FFM, leg SMM, arm FFM, trunk FFM	↓ ALM, leg FFM, leg SM ≈ whole-body FFM, arm FFM, trunk FFM
(Knudsen <i>et al.</i> 2012)	9 young men (24 (3) years) Overfed to positive energy balance of 1978 (146) kcal/day	14 days 10,278 (715) to 1,521 (131)	DXA; total FFM	≈ total FFM
(Krogh-Madsen <i>et al.</i> 2010)	10 young men (24 (2) years)	14 days 10,501 (808) to 1,344 (33)	DXA; trunk LM, arm LM, leg LM	↓ leg LM ≈ trunk LM, arm LM
(Walhin <i>et al.</i> 2013)	26 young men (25 (7) years) <i>Two groups receiving either 50% surplus kcal daily, or 50% surplus kcal and 45 min running daily</i>	7 days 12,562 (3,520) to 3,672 (866) 10,544 (756) to 3,690 (400) for groups respectively	DXA; whole-body LM	↑ whole-body LM in both groups

Abbreviations: DXA, dual energy X-ray absorptiometry; FFM, fat free mass; ALM, appendicular lean mass; SMM, skeletal muscle mass; LM, lean mass; SR, step-reduction only limb; SR+RT, step-reduction with resistance training limb, SMI; skeletal muscle index

Table 1.1 continued. Studies using step-reduction models with assessment of changes in muscle mass

Reference	Subjects	Step-reduction (Duration and steps/day)	Measures of muscle mass reported	Results
(Devries <i>et al.</i> 2015)	30 older men (70 (1) years) <i>Three groups undertaking three sessions/week of unilateral low-load resistance training with nutritional interventions</i>	14 days 7,714 (809) to 1,288 (62) 7,119 (797) to 1,270 (88) 6,273 (981) to 1,161 (107) for groups respectively	DXA; total FFM, ALM, leg FFM, leg SMM	↓ SR leg FFM ↑ SR+RT leg FFM and SMM ≈ total FFM, SR leg SMM (3 groups pooled for muscle mass data)
(McGlory <i>et al.</i> 2017)	22 overweight, pre-diabetic older adults; 10 women (69 (4) years)	14 days 7,362 (3294) to 991 (97)	DXA; total-body FFM, ALM, leg LM, SMI	≈ total-body FFM, ALM, leg LM, SMI

Abbreviations: DXA, dual energy X-ray absorptiometry; FFM, fat free mass; ALM, appendicular lean mass; SMM, skeletal muscle mass; LM, lean mass; SR, step-reduction only limb; SR+RT, step-reduction with resistance training limb, SMI; skeletal muscle index

In young males (27 (6) years) who reduced activity from >10,500 to \approx 1,350 steps/day for 14 days Krogh-Madsen *et al.* (2010) observed a 2.8% reduction in lean leg mass measured by DXA. The authors described this finding as unexpected based on the view that muscle atrophy is likely to occur only in more dramatic forms of unloading. Further data from this study also revealed significant increases in mean intra-abdominal fat mass of the group from 693 mL to 740 mL (Olsen *et al.* 2008). To date, only one study has used this model to directly investigate muscle wasting and anabolic resistance in healthy elderly individuals (Breen *et al.* 2013). As may have been expected based on previous data comparing older to younger participants in bed rest studies, Breen *et al.* (2013) found markedly more pronounced losses in muscle mass in 10 men and women aged 66-75 years walking <1,500 steps/day for 14 days. The \approx 76% reduction in daily stepping in this study resulted in a 3.9 % loss of leg skeletal muscle, which is 25% greater than the losses observed in younger individuals undertaking \approx 88% reduction in daily stepping (Krogh-Madsen *et al.* 2010). However, in the only other published study to date employing the 14-day step-reduction model in older adults without the use of any protective intervention, overweight and pre-diabetic adults (aged 69 (4) years) displayed no loss of lean tissue (McGlory *et al.* 2017). The differences in lean tissue mass prior to step-reduction was highlighted as a potential reason for the divergent findings from the previously reported loss of lean mass by Breen *et al.* (2013). The suggestion was that the healthy older adults may have had more muscle mass to lose than their pre-diabetic counterparts, and were thus predisposed to greater muscle atrophy with reduced activity (McGlory *et al.* 2017). Another potential explanation for differing degrees of muscle mass loss with step-reduction may be the relative reduction of steps by the groups in these studies may be of note; the healthy older participants had been taking <6,000 steps/day before the intervention (Breen *et al.* 2013), less than half in comparison to the healthy younger participants (Krogh-Madsen *et al.* 2010). Equally, despite being less metabolically healthy, the overweight adults in the study of McGlory *et al.* (2017) habitually took 1,500 more steps per day than the healthy older adults in study by Breen *et al.* (2013). It may be that lower levels of habitual physical activity in the older group predisposed those participants to greater loss of muscle tissue compared to the younger group, and partly explain differences in muscle loss compared to the pre-diabetic group. This may support the concept of habitually higher levels of physical activity eliciting a protective effect against anabolic resistance and therefore muscle wasting induced by

short episodes of reduced activity. However, Krogh-Madsen *et al.* (2010) observed no correlation between the decline in number of steps from baseline and loss of lean leg mass between their younger participants. The currently limited body of evidence leaves it unclear as to whether the substantial muscle mass loss observed by Breen *et al.* (2013) was in fact a result of ageing *per se* rather than lower habitual physical activity prior to step reduction.

It seems a feature of reduced activity models by step-reduction in older adults is a decreased rate of MPS. Despite no reductions in whole body lean mass, integrated rates of muscle protein synthesis assessed by orally ingested deuterated heavy water tracer were reduced by 12% with two-weeks of step-reduction to <1,000 steps/day in overweight older adults (McGlory *et al.* 2017). Furthermore, Breen *et al.* (2013) reported MPS response to provision of 25 g of egg protein to be reduced by $\approx 26\%$ following inactivity compared to pre-intervention, however there were no reductions in the associated signalling pathways (mechanistic target of rapamycin complex 1 (mTOR), phospho-70 kDa S6 protein kinase (p70S6K), and eukaryotic initiation factor 4E binding protein (4E-BP1) from pre-to-post-step-reduction as seen in bed rest studies with older individuals (Drummond *et al.* 2012). Whilst this requires further examination, it may have been due to biopsy sample timing (four hours post-feeding); this was likely after peak phosphorylation which is generally considered to occur between one and two hours post-feeding (Atherton *et al.* 2010). Alternatively, if anabolic signalling pathways were not down-regulated then other mechanisms could be explored, such as impaired amino acid transporter activity at skeletal muscle as observed due to bed rest in older adults by Drummond *et al.* (2012).

An important consideration from both Breen *et al.* (2013) and McGlory *et al.* (2017) was that no changes were elicited by two weeks of step reduction in isometric knee extensor torque of older adults. Previously, 10 days of bed rest in healthy older adults that resulted in 6.3% losses in mean lean leg tissue brought about a concomitant drop in mean isokinetic knee extensor strength of 15.6%, but ranging up to 23.1% (Kortebein *et al.* 2007). As the methodology of strength measurement was essentially the same, it would be expected that accompanying muscle mass losses of nearly two thirds of that observed by Kortebein *et al.* (2007) there would be at least some measurable loss of muscle strength due following the step reduction. Further investigation, possibly with different strength and functional measurement tools is

warranted from this finding given that strength in older individuals commonly deteriorates at a greater rate than mCSA (Seene and Kaasik 2012), and particularly in circumstances of muscle unloading (Hackney and Ploutz-Snyder 2012). Nonetheless, it may have been that even the limited amount of ambulation permitted may have provided some protective effect against the loss of strength over the two week intervention.

1.3.5 Possible mechanisms for exaggerated disuse induced muscle loss with ageing.

Reviews by Bodine (2013) and Mallinson and Murton (2013) give detailed accounts of the current understanding of molecular mechanisms and signalling pathways involved in altering the balance of muscle protein turnover in response to disuse that results in muscle wasting. Though some of the primary mechanisms recognised in the literature will be highlighted here, the emphasis will be on drawing attention to how these mechanisms are altered by ageing induced changes in human physiology that may predispose older individuals to increased sensitivity to muscular disuse.

The total unloading models used in previous studies have allowed exploration of processes by which muscle protein turnover changes result in marked net muscle protein loss over time. To briefly summarise these findings, it has long been established that in humans the rate of MPS at rest falls rapidly at the onset of disuse and remains depressed until mechanical loading is reintroduced (Gibson *et al.* 1987). Thus far, fewer studies have addressed in vivo MPB rate responses to unloading due to the methodological complexities involved, particularly with regards to identifying the protein origin of tracer-labelled amino acids measured in arterial, venous, and intracellular sites. There is little direct evidence for elevation of MPB with unloading, however specific genes coding for skeletal muscle atrophy have been shown to be upregulated during bed rest. Expression of Muscle Ring Finger 1 (MuRF1) and Muscle Atrophy F-box (MAFbx) is hypothesised to regulate proteasome-mediated degradation of muscle proteins during atrophy, and temporal elevations have been reported under conditions of lower limb muscle unloading by Jones *et al.* (2004) and de Boer *et al.* (2007). A transient increase in MPB may be supported by data from Wall *et al.* (2014b) who reported marked elevations in mRNA expression of MAFBx and MuRF1 in a group undergo five days of limb immobilisation, compared with MuRF1 not being elevated after 14 days of immobilisation in a separate group. Generally, changes in MPB are not observed with unloading; for example Ferrando *et al.* (1996) did not observe any change in MPB rate across 14 days of bed rest, when measuring both whole body and skeletal muscle protein metabolism. However, Tesch *et al.* (2008) reported a transient increase in MPB lasting ≈ 72 -hours post onset of

unloading, measured with a 3-methylhistidine microdialysis technique (3MeHis). The interpretation of data and methodology of this study have been respectfully criticised, in particular the choice of glucose as a marker of 3MeHis recovery and no direct measure of blood flow around the dialysis probe likely rendering this finding unreliable (Rennie *et al.* 2008). As in younger individuals, there is a lack of evidence for an associated increase in MPB playing a substantial role in disuse induced muscle wasting in older individuals (Drummond *et al.* 2012; Rennie *et al.* 2010). Overall, the dominant mechanism inducing atrophy during muscle disuse is still a matter of debate, as has been highlighted in recent discussions in the literature (Phillips and McGlory 2014; Reid *et al.* 2014).

During periods of normal physical activity, MPS is observed to increase markedly above basal levels following amino acid ingestion (Bohe *et al.* 2003; Symons *et al.* 2009). Reduced MPS at rest and in response to stimuli normally considered anabolic appears to be the driver of unloading induced muscle atrophy. Basal MPS accounted for almost half of the loss in muscle mass during 14-days of unilateral knee immobilisation in young men and women, and there was a blunted MPS response to amino acid infusion in the immobilised leg compared to the non-immobilised leg (Glover *et al.* 2008). It is thought that the combination of ‘anabolic resistance’ to protein feeding and the decreased basal MPS has a great enough influence on the balance of muscle protein turnover that any change in the rate of MPB does not play a substantial role in disuse induced muscle mass loss (Phillips *et al.* 2009; de Boer *et al.* 2007; Gibson *et al.* 1987; Glover *et al.* 2008).

The roles of mTOR (Hall 2013) and p70S6K in up-regulation of protein translation initiation and protein synthesis in human skeletal muscle are well accepted (Rennie *et al.* 2004). Evidence from Cuthbertson *et al.* (2005) implicates suppressed expression and activation of these recognised anabolic signalling pathways in anabolic resistance in older individuals. Three hours after ingestion of 10 g of essential amino acids the phosphorylation of mTOR and p70S6K only increased 2.7- and 3.5-fold respectively in older individuals, compared to 5.2- and 8.1-fold in younger individuals (Cuthbertson *et al.* 2005). Diminished anabolic signalling is further exaggerated by inactivity in older individuals (Drummond *et al.* 2012), despite immobilisation studies using young participants not demonstrating any decrease in mTOR pathway signalling (Bodine *et al.* 2001). Drummond *et al.* (2012) identified that after seven days of bed

rest the mTOR signalling pathway had a blunted response to essential amino acids (EAA) ingestion accompanied by significantly less MPS three hours after EAA ingestion compared to pre-bed rest in older individuals. An earlier study showed that older individuals (70 (5) years) also exhibited anabolic blunting in response to resistance exercise; MPS expressed as total protein synthesised in the four hours following unilateral leg extension exercise was $\approx 30\%$ lower compared to younger individuals (Kumar *et al.* 2009). Again, significantly lower phosphorylation of the downstream effectors of mTOR (p70S6K and 4E-BP1) were observed in the older compared to the younger group one hour after exercise of an equal volume across a range of intensities above 60% of one repetition maximum.

1.4 Protective interventions

1.4.1 Exercise to attenuate step-reduction induced atrophy and function loss.

In the context of muscle unloading research simulating space flight, resistance exercise has been investigated as a countermeasure to disuse atrophy (Trappe *et al.* 2007). The concept of re-introducing bouts of exercise whilst concomitantly just reducing physical activity, for example with step-reduction models, has been studied far less. In a study by Walhin *et al.* (2013), running was implemented during a period of step-reduction, albeit with the primary aim of protecting against negative changes in insulin sensitivity and adipose tissue gene expression in the face of reduced activity and overfeeding in young healthy individuals. Twenty six healthy young males (25 (7) years) randomised to two parallel groups took <4,000 steps per day for one week alongside overfeeding by 50% of habitual daily energy intake, with one of the groups also performing 45 minutes/day of running at 70% of maximal oxygen uptake with additional overfeeding to maintain 50% daily energy intake. Interestingly, DXA measured increases in lean tissue were observed following one week of step-reduction to <4,000/day in both groups; 2.6 (95% CI; 1.9-3.4) kg in the group just receiving overfeeding with step reduction, and 1.0 (95% CI; 0.1-2.0) kg in the group additionally performing the daily exercise. The authors acknowledge that this is almost certainly due to muscle glycogen storage and accompanying water retention in muscle in response to overfeeding, which DXA would non-discriminately identify as lean tissue. As a concept however, the re-introduction of daily exercise was effective in countering the effects of the reduced activity and energy surplus. More recently, Devries *et al.* (2015) explored the effects of introducing six bouts of low-load unilateral resistance exercise (three sets of leg press and leg extension to volitional failure at 30% of 1-RM) during 14 days of step-reduction in older men and demonstrated remarkable protective effects against muscle atrophy compared to the non-exercised limb. This study undertook a three group design in which various combinations of daily nutritional supplements and test beverages for an infusion trial examining myofibrillar fractional synthetic rate (FSR) were provided to groups. These conditions were either 5 g glycine per day, with 20 g whey protein isolate and 15 g glycine as test beverage; 5 g glycine per day, with 20 g micellar whey protein and 15 g glycine as test beverage; or 5 g citrulline per day, with 20 g micellar whey protein and 5 g citrulline as test beverage. Moreover, no differences were observed in muscle mass changes between

groups, so these data were collapsed and demonstrated that whilst participants non-exercised leg lost 1.4% skeletal muscle mass, the exercised leg in fact increased leg skeletal muscle mass by 1.4% (Devries *et al.* 2015). The authors attribute the smaller loss of leg lean tissue observed in this study compared to that of Breen *et al.* (2013) to a potential cross-education effect of training the contralateral limb, although it is not clear whether the nutritional supplementation may have played a role in this attenuation of muscle atrophy. However, again step-reduction alone did not induce measureable loss of muscle strength, and more evidence of the potential cross-education effect of unilateral limb training comes from an increase in knee extension 1-RM in the untrained as well as trained limb (Devries *et al.* 2015).

The study of Devries *et al.* (2015) provides encouraging evidence that in real world scenarios of reduced activity periods, even relatively moderate exercise might be effective for attenuating loss of muscle mass and function. However, this is a somewhat paradoxical concept, because of course if an individual were to be performing exercise then they would not be in a state of 'disuse'. Therefore, to frame this more appropriately, in circumstances in which reduced daily activity is unavoidable but capacity to exercise is not compromised, short bouts of exercise may be sufficient to replace habitual daily physical activity in maintaining muscle size and function. In this vein, if an exercise regime is to be feasible during a period of reduced activity, it is likely to need to be homebased. Of the 121 trials on progressive resistance training for improving physical function in older adults included in the most recent Cochrane Review on the topic, only 10 implemented exclusively homebased exercise (Liu and Latham 2009). Moreover, of these 10 studies, all examined either disabled, frail, or mobility limited participants, and exercise regimes required some form of equipment. A systematic review of studies specifically of homebased exercise in older adults by Thiebaud *et al.* (2014) highlights that homebased exercise regimes tend to replicate traditional gym based training session structures of multiple sets of a specific repetition numbers, over a range of exercises, training 2-3 times/week. Adherence to homebased exercise is often low, and this may be due to the time or equipment required to carry out the training (Thiebaud *et al.* 2014).

The aim of an exercise intervention during a period of step-reduction would be attenuate loss rather than specifically induce hypertrophy or strength gains, and as such an alternative structure to a training programme to account for this to maximise

adherence. Response to resistance training is deemed to be quick in adults that have little resistance training experience (Ratamess *et al.* 2009). As such, even modest training stimuli may be sufficient in inducing protective benefits during reduced activity, or even induce gains in strength in generally non-active older adults (Chandler and Hadley 1996). Moreover, training with light loads has been demonstrated to increase MPS in older men (Agergaard *et al.* 2017). Dankel *et al.* (2016) make a convincing case that increasing training session frequency, accounted for by reducing session load, might be a way to optimise hypertrophic responses to a training programme. This notion is supported by (Morán-Navarro *et al.* 2017) who have shown that reducing resistance training session load, in particular not completing repetitions to failure, allows for shorter recovery time. Undertaking very short bouts of daily homebased exercise might be an efficient means attenuate muscle loss in older adults during periods of reduced activity. This model may even be suitable to improve muscle function, and potentially delay sarcopenia in older individuals who are still mobile and capable of a range physical activity, yet currently do not undertake any form of exercise (Witard *et al.* 2016).

1.4.2 Nutritional strategies to attenuate muscle loss with ageing

The likely causes of the continued loss of muscle size with ageing, and therefore to some extent strength, are becoming more clearly understood (Fragala *et al.* 2015). As previously mentioned, a key contributor to sarcopenia is anabolic resistance, i.e. symptomatically reduced MPS response to amino acid feeding, resulting in a negative mismatch between overall protein synthesis and degradation (Burd *et al.* 2012). However, this is a somewhat plastic state that can be overcome by manipulating diet and performing resistance exercise, thus representing a crucial target to stem progression of sarcopenia (Moore 2014; Symons *et al.* 2011; Dideriksen *et al.* 2013).

Simply increasing the protein content of a meal may overcome the challenge of anabolic resistance (Paddon-Jones *et al.* 2015). Moore *et al.* (2015) demonstrated with biphasic linear regression of a large data set of MPS rates to protein portion sizes from five studies undertaken at McMaster University, that 0.40 g/kg total body mass or 0.61 g/kg of lean body mass is sufficient to saturate MPS rate in older individuals. This MPS rate in response to ingestion of sufficient amounts of protein matched the rate achieved by younger males ingesting 0.24 g/kg body mass. For reference, these data suggest that ingestion of ≈ 28 g of protein would achieve maximal protein synthetic rate per meal in a 70 kg older male. Indeed Symons *et al.* (2009) found no impairment in the mixed muscle FSR following provision of 113 g of lean beef (containing 30 g of protein) in older individuals compared to younger individuals. Furthermore, provision of 340 g of lean beef (90 g protein) had no further anabolic effect of mixed muscle FSR in either older or younger individuals. Rather than suggesting that anabolic resistance does not exist in older individuals, these studies highlight the importance of large doses of protein to stimulate MPS.

The logical progression from this is to increase the post-prandial time spent at high or maximal rates of MPS by ensuring that every meal consumed by older adults contains sufficient protein (Breen and Phillips 2011), however, this is as yet not experimentally supported with long term studies (Mitchell *et al.* 2014). In any case, with the auxiliary benefits of protein ingestion such as increased satiety (Gosby *et al.* 2013), adding protein to meals that traditionally aren't protein rich, such as breakfast (Paddon-Jones *et al.* 2015; Cardon-Thomas *et al.* 2017), may be a pragmatic

manipulation of diet for older adults. A final important consideration for protein feeding of older adults besides size and timing of the protein portion is the quality of the protein (i.e. the amino acid profile). The amino acid leucine plays a vital role in stimulating muscle protein synthesis when fed in quantities of 2.5-5 g per meal as found in typical portion sizes of dairy products such as yogurt (Casperson *et al.* 2012; Wall *et al.* 2013b; Phillips *et al.* 2015; Wilkinson *et al.* 2017).

Lastly, as alluded to previously, the other primary strategy to overcome the anabolic response is to perform resistance exercise prior to consumption of protein (Breen and Phillips 2011). Agergaard *et al.* (2017) demonstrated that high volume, low load resistance training robustly increased MPS response regardless of protein feeding intervals for the seven hours post exercise in men over 65 years of age. It seems that low load resistance exercise can enhance sensitivity of muscle tissue to aminoacidemia even during periods of very low activity which would otherwise be considered catabolic. During 14-days of step-reduction to 1,500 steps/day, six low load unilateral resistance training sessions actually induced hypertrophy in older men, to the extent that a measurable difference in leg lean mass between trained and untrained legs was observed (Devries *et al.* 2015). With resistance exercise priming muscle to exhibit a more marked MPS response to protein feeding for at least 24-hours post exercise, older adults may benefit from daily resistance exercise bouts (Burd *et al.* 2011).

1.5 Thesis aims

1. Investigate the relationship between habitual physical activity and power and force generating capacity using a pneumatic leg press, in healthy, active older adults.
2. Explore the efficacy of a homebased exercise programme implementing short bouts of exercise on a daily basis to improve leg muscle function and anthropometric characteristics, in healthy, non-exercising older adults.
3. Implement the aforementioned homebased exercise regime as a countermeasure to the negative consequences of two-weeks of step-reduction in healthy older adults. Specifically, the effects of the exercise intervention were compared against the effects of step-reduction alone, and the responses to a two-week retraining programme were examined.

Chapter 2: Habitual physical activity, and leg power and strength in healthy, active older adults- A cross-sectional study

2.1 Introduction

The loss of muscle mass (sarcopenia) and strength (dynapenia), particularly in the lower limbs, are regarded as central causes of functional decline and subsequent loss of independence in older adults (Walston 2012; den Ouden *et al.* 2013). It is evident that age related muscle loss is associated with an uncoupling of the relationship between muscle size and force generating capacity that would normally be observed in younger healthy individuals (Fukunaga *et al.* 2001; Goodpaster *et al.* 2006). Furthermore, maximum contraction speed of muscle is also observed to decrease with advancing age (Thom *et al.* 2007). As such, the rate of force transfer from muscle (i.e., power) is lost to a greater extent than maximal force producing capacity alone (Izquierdo *et al.* 1999; Clark *et al.* 2013). Power is considered a better predictor of functional status than muscle mass or strength alone, and is more sensitive to age-related physiological changes such as impaired neuromuscular activation and changes in muscle architecture (Cooper *et al.* 2013; Bean *et al.* 2002). The use of pneumatic resistance exercise machines instrumented to record kinetic data has emerged as an accurate, valid and reliable tool to assess lower limb muscle power (Reid and Fielding 2012).

Maintaining an adequate level of physical activity is regarded as crucial for preserving muscle function into later years of life (Manini and Pahor 2009). However, at the whole body level, the relationship between habitual physical activity and muscle function in healthy older adults is surprisingly unclear. Some studies demonstrate associations between leg power and daily moderate intensity physical activity level, independent of lower limb muscle mass or resistance training (Straight *et al.* 2016). Others demonstrate associations between daily physical activity level and some indices of muscle function but not others. For example, Morie *et al.* (2010) observed in mobility-limited older men that leg press strength was weakly correlated with waist mounted accelerometer measured physical activity level, but leg press power was not. Conversely some studies report no correlation between physical activity and muscle function, even when considering changes over time. No association was found between changes in isokinetic knee extensor strength and physical activity over 10

years in a wide range of physically functioning older men and women (Hughes *et al.* 2001). Overall, current understanding of the relationship between habitual physical activity levels and lower limb muscle power in active, healthy older individuals is incomplete.

The aim of the present study was to compare characteristics of muscle function (maximal velocity, force, and power) during bilateral leg press exercise between healthy older and younger individuals and to explore relationships between muscle function characteristics and habitual physical activity using multidimensional physical activity monitoring. It was hypothesised that older adults would produce less force and power than younger adults, both in absolute terms and relative to muscle size. It was also hypothesised that there would be no association between leg pressing force and power capabilities, and older adults habitual physical activity levels (consider as a ratio of total energy expenditure (TEE) to basal metabolic rate (BMR), and minutes per day spent sedentary or engaging in moderate to vigorous activity (MVPA)).

2.2 Methods

2.2.1 Participants

Healthy, older (65-80 years) and younger (20-35 years) men and women were recruited for the study through local newspaper advertisement and social media. Potential participants were fully informed as to the study design and methodology with written information, and an informal telephone interview was conducted by a member of the research team to ascertain initial eligibility. Individuals who were non-smokers, body mass index (BMI) $<30 \text{ kg/m}^2$, not regularly taking anti-inflammatory medication, and had no contraindications to exercise or recent history of musculoskeletal injury were invited to the laboratory to undertake the Short Physical Performance Battery (SPPB) (Guralnik *et al.* 1994). Individuals scoring 8 or above, and not scoring zero on any particular SPPB test, were included in the study. All participants provided written informed consent. The protocol was approved by the University of Bath Research Ethics Approval Committee for Health. Participant characteristics are presented in Table 2.1.

Table 2.1. Participant characteristics.

	Older ($n = 50$; ♀ = 25)	Younger ($n = 30$; ♀ = 15)
Age (years)	70 (4)**	25 (4)
Body Mass (kg)	69.3 (12.4)	68.5 (10.8)
BMI (kg/m^2)	24.3 (3.4)*	22.6 (2.8)
VL muscle thickness (mm)	18 (4)**	22 (4)
SPPB Score (/12)	11 (1)	11 (1)

Note. Data presented as mean (standard deviation). BMI = Body mass index; VL = *vastus lateralis*. SPPB = Short Physical Performance Battery. */**denotes significant differences between age groups (* $P < 0.05$, ** $P < 0.01$).

2.2.2 Procedures

Participants visited the laboratory on two occasions separated by at least seven days. Participants were requested to refrain from exercise in the 24 hours prior to testing, and to continue with their normal dietary habits. During the first visit, measurements of *vastus lateralis* (VL) muscle thickness were taken using ultrasound. Transverse images were taken at 50% of thigh length from the left leg using a 128 element linear array transducer (LV7.5/60/96Z, TELEMED, Lithuania) operating in B-mode at 8.0 MHz. Three images were taken from each participant. Muscle thickness was measured adjacent to the muscle fascia at the thickest part of muscle belly between the inside edges of the muscle fascia in each of the images and a mean calculated for each participant. All images were processed by the same researcher (OJP) using ImageJ software (1.44p, Wayne Rasband, National Institutes of Health, USA), and each image analysed three times with absolute typical error (95% confidence intervals) of 0.18 (0.16-0.21) mm (Hopkins 2015). See Figure 2.1 for an example ultrasound image demarcated with muscle thickness.

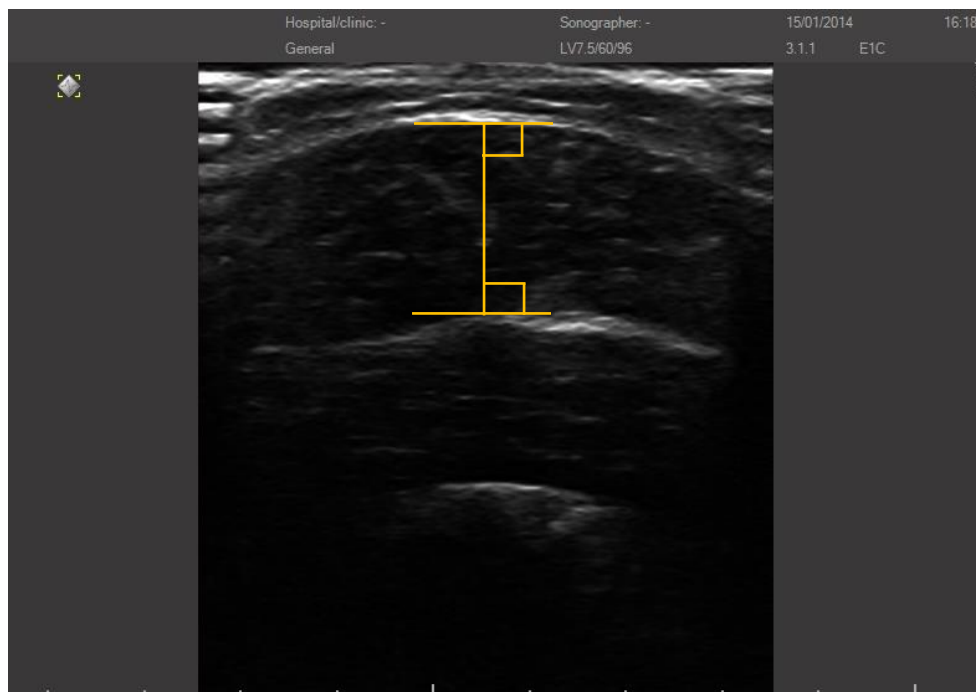


Figure 2.1. Example ultrasound image of a vastus lateralis muscle with lines indicating the distance taken as muscle thickness.

2.2.3 Force and power

A Keiser A420 seated pneumatic leg press dynamometer (Keiser®, Fresno, CA) was used to measure lower limb force and power production characteristics using the manufacturer's software (see Figure 2.2). The seat position was set such that pre-repetition knee angle was approximately 90° and the participant was comfortable. The same seat position was used in both trials. During the preliminary trial, participants performed a 1-RM test in which discrete repetitions to failure were attempted at participant-selected increments in resistance. Repetition velocity and rest periods between repetitions were self-selected, with participants instructed to aim to reach their 1-RM within 20 repetitions. Ten minutes of seated rest were then observed, followed by familiarisation with an incremental power test in which approximately 10 discrete leg press repetitions were performed at maximum voluntary movement velocity for each repetition. Resistance and duration of rest between efforts increased with each repetition and were calculated by the manufacturer's software, with resistance of the tenth repetition corresponding with the previously achieved 1-RM. For the main trial the same incremental power test was used with the same set of increasing resistances. Following 10 minutes of seated rest, participants undertook a test of eight discrete leg press repetition of maximum velocity at 40% of the 1-RM achieved in the familiarisation, with 60 seconds of rest between repetitions.



Figure 2.2. The Keiser A420 seated pneumatic leg press dynamometer (Keiser®, Fresno, CA) used to measure leg pressing force and power, with the participant in the 'start' position (left) and 'finish' position (right) of a single repetition.

Sampling frequency of linear displacement and pressure within the pneumatic pistons (creating resistance at the foot pedals of the leg press machine) was 400 Hz, with pedal velocity (m/s) and resistance force (N) calculated by the manufacturer's software. Data were sampled independently from each leg, and the mean velocity, force and power from both legs for each repetition used for subsequent analysis.

Figure 2.3 displays a typical force trace for a single repetition, with only the left leg included for clarity. These data were sampled for the duration of the concentric phase of each discrete repetition of the incremental power test, with the first and last 5% of pedal displacement discarded from analysis by the manufacturer's software. This software selected analysis phase is depicted by the thick pink line on Figure 2.3.

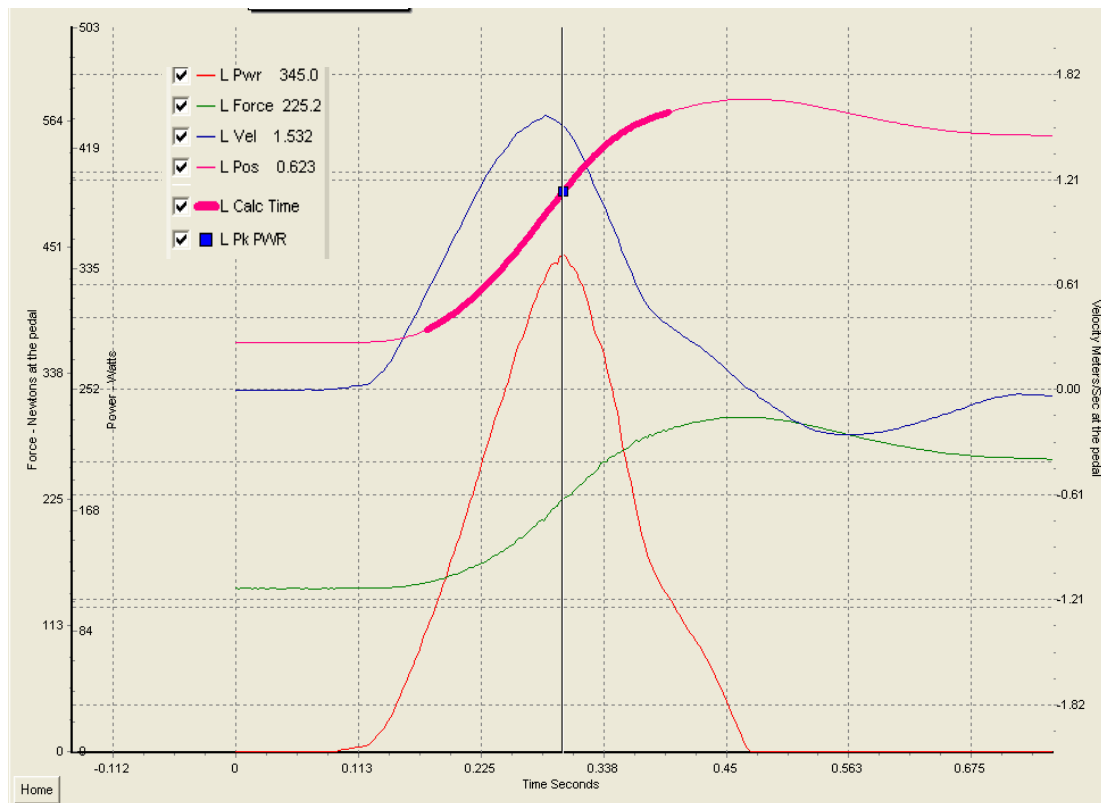


Figure 2.3. A representative force trace from a single repetition on the Keiser A420 leg press. Pedal displacement position is represented by the thin pink line, pedal velocity by the thin blue line, force by the thin green line, and power by the thin red line. The thick pink line indicates the time phase that raw data is analysed (excluding first and last 5% of pedal displacement). The small blue square demarks the instant of peak power, with the vertical black bisecting line from the x-axis indicating the instant of peak power relative to the velocity-time and force-time traces.

Leg press resistance was provided by pneumatic pistons, thus resistance increased across the range of motion of each repetition. This relationship between pedal displacement and force is shown in Figure 2.3; the thin pink line representing pedal position, and the thin green line representing force, calculated from pressure within the compressed piston. As such, peak force of a repetition was dependent on pedal displacement. Consequently velocity and force at the instant of peak power were recorded for each repetition rather than peak velocity and peak force. On Figure 2.3, power across the repetition is represented by the thin red line, and the instant of peak power is marked by small blue square on the pink line. The vertical line from the x-axis passing through the instant of peak power emphasises the location on the velocity-time trace (thin blue line) and force-time trace (thin green line) that the discrete data points for velocity and force at the instant of peak power for each repetition would be taken. Data were subsequently analysed manually using Microsoft® Excel® 2013 to determine theoretical maximum force and velocity, as proven by Figures 2.4 and 2.5.

Figure 2.3 further emphasises that the muscle contraction required to complete a repetition on a pneumatic resistance exercise machine cannot be described as truly isotonic, as addressed in the section 1.1.4 of the Introductory Chapter 1. In contrast to a true isotonic contraction whereby a constant force is applied across a range of motion (Frontera and Ochala 2015), the nature of resistance created by a pneumatic piston in fact inherently increases the required force across the range of motion. The type of contraction demanded by a pneumatic resistance machine has not been classified with a specific nomenclature, however for the purpose of the present thesis, it will be referred to as a dynamic contraction. Indeed, it is not possible to perform a true isotonic contraction of muscle fibres through movements that occur at the whole body level in any case, due to the elastic properties of tendons that connect muscle to the skeleton. Whilst the paradigm of isotonic contractions presents a clear metric by which to measure change in muscle fibre contractile properties, it is clear the leg pressing at the whole body level cannot fit within that paradigm. This is further evidenced by the fact that the single muscle fibre force-velocity relationship is curved as per the equations of Hill (Hill 1964; Hill and Sec 1938), whereas Bobbert (2012) demonstrated that the force-velocity relationship of a leg pressing movement is quasi-linear. The likely reasons that the force-velocity relationship of a leg pressing movement is not curved, as would be seen in a single fibre, is due to a complex combination of multiple muscle

group coordination, neural properties, and mechanical properties of muscle and tendon units. Nonetheless, the characteristics of the linear relationship of force and velocity, such as the theoretical intersects of velocity at zero external force, and force at zero velocity, can be established. Furthermore, power can be calculated from the force-velocity relationship. Whilst these variables cannot be said to describe the behaviour of specific muscles, they do characterise the lower limb as whole, which is perhaps of greater real world relevance to an individual.

As briefly mentioned previously, with peak force of a discrete repetition of the leg press dictated by range of motion, repetitions could not be characterised by force alone, as might be the case if using a traditional loading of a movement, such a weight stack machine. Furthermore, an average of the force generated during a repetition would be inherently biased by lower limb length between participants. Equally, the impact of the unusual characteristic of increasing load across a repetition range of motion on velocity across a range of motion has not been investigated. As such, a common time point of the instant of peak power of each repetition was used to define the instant at which a discrete force and velocity could be recorded. A linear regression of velocity and force at peak power for each repetition across the incremental power test was calculated, and maximum theoretical velocity (V_{\max}) and force (F_{\max}) were extrapolated (see Figures 2.4 and 2.5).

Previous studies of muscle function characteristics *in vivo* have often drawn data from participants performing a series of discrete repetitions of an exercise at maximum contraction velocity against increasing external loads (Foldvari *et al.* 2000; Macaluso and De Vito 2003; de Vos *et al.* 2005). Peak power would then be defined as the greatest power achieved in any of the performed repetitions irrespective of the % of 1-RM at which the repetition was performed (Bean *et al.* 2002). However, this approach may lack resolution with peak power likely occurring at an external load between resistances actually attempted. Therefore interpolated peak power (P_{\max}) was determined by numerical differentiation of the second-order polynomial equation calculated from the force-power profile (Samozino *et al.* 2012). See Figure 2.4 for a method validation proof from example data from an incremental power test, and Figure 2.5 for example F_{\max} and P_{\max} curves generated from the aforementioned example data. For analysis of the eight repetition test, the peak power of eight discrete repetitions was recorded, and the mean calculated.

Rep #	Peak power (PWR)		Pos @ peak PWR		Vel @ peak PWR		Force @ peak PWR	
	Left	Right	Left	Right	Left	Right	Left	Right
1	482	480	0.65	0.68	1.93	1.84	250	261
2	552	559	0.64	0.67	1.84	1.79	300	313
3	605	602	0.64	0.65	1.66	1.67	364	360
4	691	697	0.64	0.65	1.60	1.62	431	429
5	729	752	0.63	0.65	1.53	1.53	477	492
6	779	793	0.64	0.66	1.41	1.40	551	566
7	806	828	0.62	0.64	1.32	1.33	608	623
8	854	870	0.62	0.65	1.28	1.24	670	702
9	830	878	0.60	0.62	1.16	1.21	714	728
10	778	875	0.62	0.63	0.98	1.10	797	795
11	712	773	0.61	0.64	0.85	0.88	843	881
12	691	750	0.62	0.64	0.74	0.79	930	945
13	562	557	0.62	0.64	0.57	0.55	991	1012
14	308	306	0.60	0.61	0.30	0.30	1020	1023
15	344	382	0.58	0.61	0.33	0.35	1043	1081
F-V slope								
	gradient	intercept						
LEFT	-0.0019	2.4536752	"=LINEST(F2:F16,H2:H16,TRUE,TRUE)"					
RIGHT	-0.0018	2.4004512	"=LINEST(G2:G16,I2:I16,TRUE,TRUE)"					
F-P curve								
	a +	bx +	cx^2					
LEFT	-0.0027	3.4086724	-248.875	"=LINEST(B2:B16,H2:H16^{1,2},TRUE,TRUE)"				
RIGHT	-0.0028	3.6578342	-334.272	"=LINEST(C2:C16,I2:I16^{1,2},TRUE,TRUE)"				

Figure 2.4. A method validation proof from example data from an incremental power test.

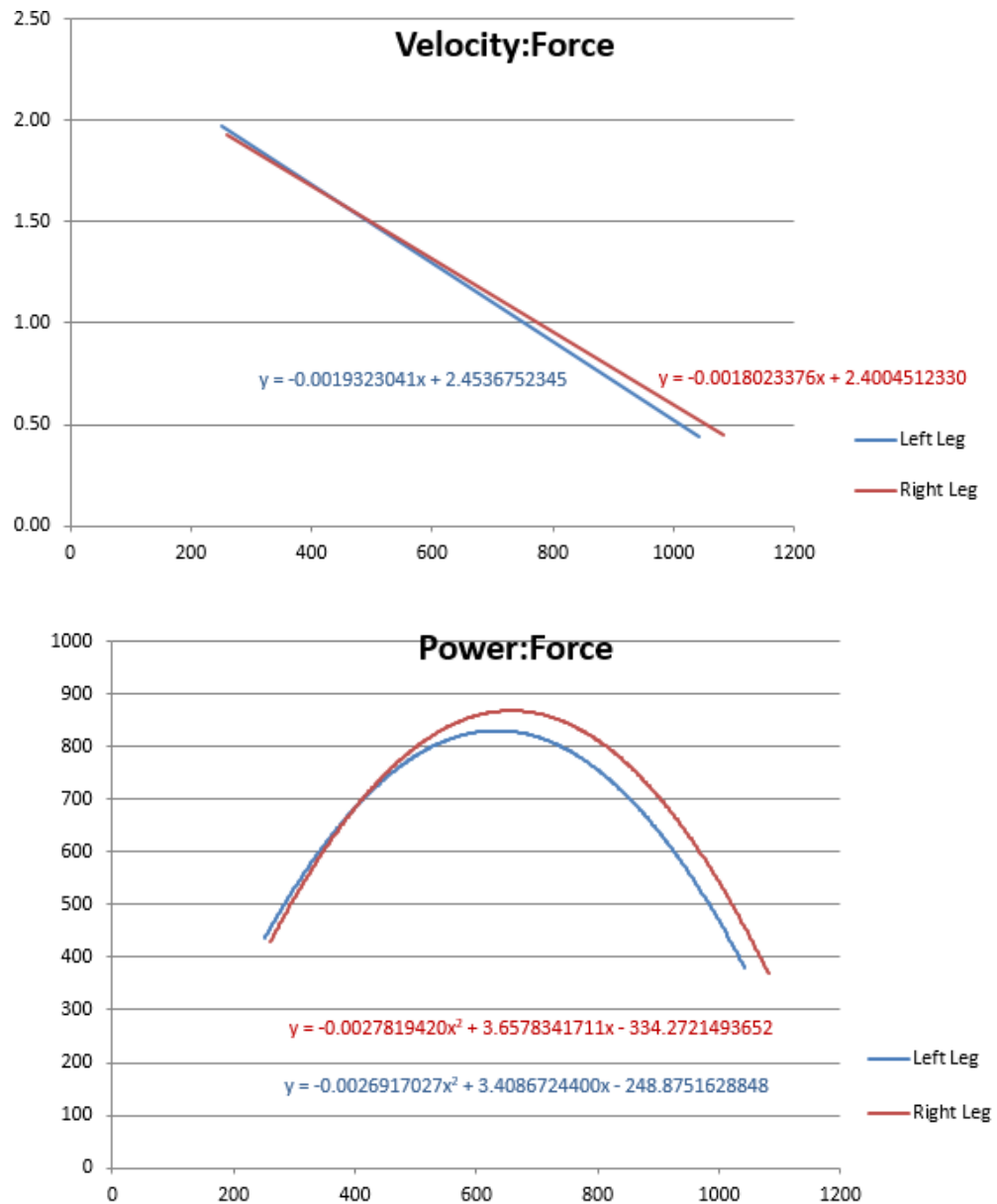


Figure 2.5 Example F_{\max} and P_{\max} curves generated from the example data presented in Figure 2.4, with corresponding equations.

As an auxiliary measurement to the incremental power test, an eight repetition test was completed, whereby participants completed eight discrete repetitions at 40% of their 1-RM, with 60 seconds rest in between. This data was collected primarily to examine intra-individual differences movement coordination variability between older and younger adults performing maximal velocity movement at deliberately sub-maximal external loads, with sufficient rest to ensure no risk of fatigue (see Wilson *et al.* (2016)). The data for the eight repetition test was included in the present thesis to

provide an additional assessment of muscle power that demonstrated that repeatability of the leg press measure without any risk of fatigue.

2.2.4 Physical activity

Participants wore a combined accelerometer and heart monitor (Actiheart™, Cambridge Neurotechnology, UK) mounted on the chest using adhesive electrode pads (3M, UK) continuously for six free-living days to record habitual physical activity in one-minute epochs using the default equations in the manufacturer's software. Participants were instructed to continue with their normal lifestyle, and to remove the monitor only for water based activity. Daily physical activity level (PAL) was calculated as a ratio of TEE to BMR, with BMR calculated using participant height, weight, age, and sex as described by Schofield (1985). Age specific physical activity thresholds were used to categorise physical activity patterns (sedentary time = ≤ 1.5 metabolic equivalents of task compared to the energy cost of sitting quietly (METs) for both groups, and for the older group MVPA = ≥ 3.2 METs, and for the younger group MVPA = ≥ 4.8 METs) (Garber *et al.* 2011).

2.2.5 Statistical analysis

All data were found to be normally distributed according to Shapiro-Wilk tests. There was no effect of sex on the differences between age groups, thus data for both sexes were pooled for each age group. Differences in anthropometric data and muscle function characteristics, PAL, and minutes per day spent within sedentary and MVPA thresholds of physical activity between age groups were assessed with independent samples *t*-test. Hedge's *g* effect sizes were calculated for between age group differences in muscle function characteristics, PAL, and minutes per day spent within given thresholds of physical activity on account of the unequal group sizes. Pearson's correlation was used to assess relationships of muscle function (V_{\max} , F_{\max} and P_{\max}) with PAL and with minutes of sedentary time and MVPA per day. All statistical analysis was conducted using SPSS v22.0 (SPSS Ins., Chicago, IL). Data are presented as mean (standard deviation) with statistical significance accepted at $p < 0.05$.

2.3 Results

2.3.1 Muscle function characteristics

As shown in Figures 2.6 and 2.7, V_{\max} and F_{\max} were significantly lower in older individuals compared to younger individuals (1.44 (0.33) m/s vs. 2.00 (0.34) m/s, $p < 0.01$, $g = 1.09$; and 1074 (310) N vs. 1615 (433) N, $p < 0.01$, $g = 0.98$ respectively). Both indices of power, P_{\max} and $P_{\max40}$, were also significantly lower in older adults (402 (165) W vs. 819 (180) W, $p < 0.01$, $g = 1.59$ (Figure 2.8); and 376 (166) W vs. 740 (271) W, $p < 0.01$, $g = 1.12$ (Figure 2.9). When F_{\max} and P_{\max} were considered relative to VL muscle thickness, $F_{\max}:\text{VL}$ was significantly lower in older individuals compared to younger individuals (60.1 (15.2) N/mm vs. 71.8 (14.7) N/mm, $p < 0.01$, $g = 0.51$), as was $P_{\max}:\text{VL}$ (22.4 (8.3) W/mm vs. 36.2 (10.3) W/mm, $p < 0.01$, $g = 0.93$) (see Figure 2.10). When F_{\max} and P_{\max} were considered relative to body mass, $F_{\max}:\text{kg}$ was significantly lower in older individuals compared to younger individuals (15.5 (3.7) N/kg vs. 23.3 (4.0) N/kg, $p < 0.01$, $g = 2.05$), as was $P_{\max}:\text{kg}$ (5.8 (2.1) W/kg vs. 11.7 (2.9) W/kg, $p < 0.01$, $g = 2.44$) (see Figure 2.11).

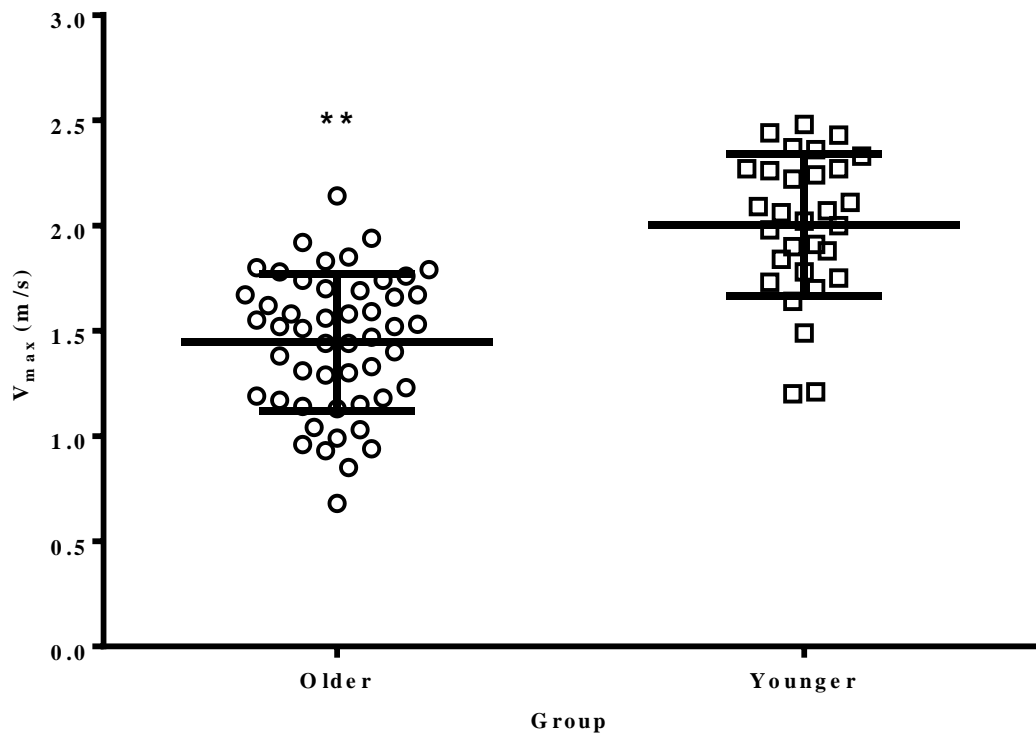


Figure 2.6. Mean \pm SD extrapolated peak velocity (V_{max}) for 65-80 vs 20-35 yr olds. **denotes significant difference between groups ($p < 0.01$).

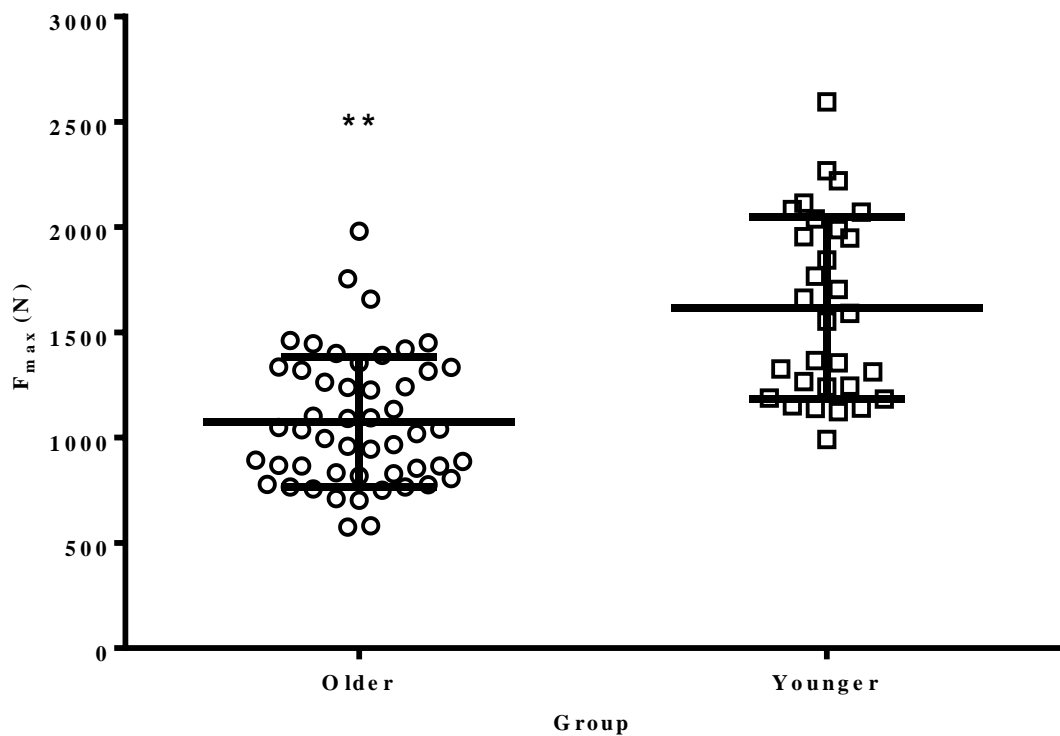


Figure 2.7. Mean \pm SD extrapolated peak force (F_{max}) for 65-80 vs 20-35 yr olds. **denotes significant difference between groups ($p < 0.01$).

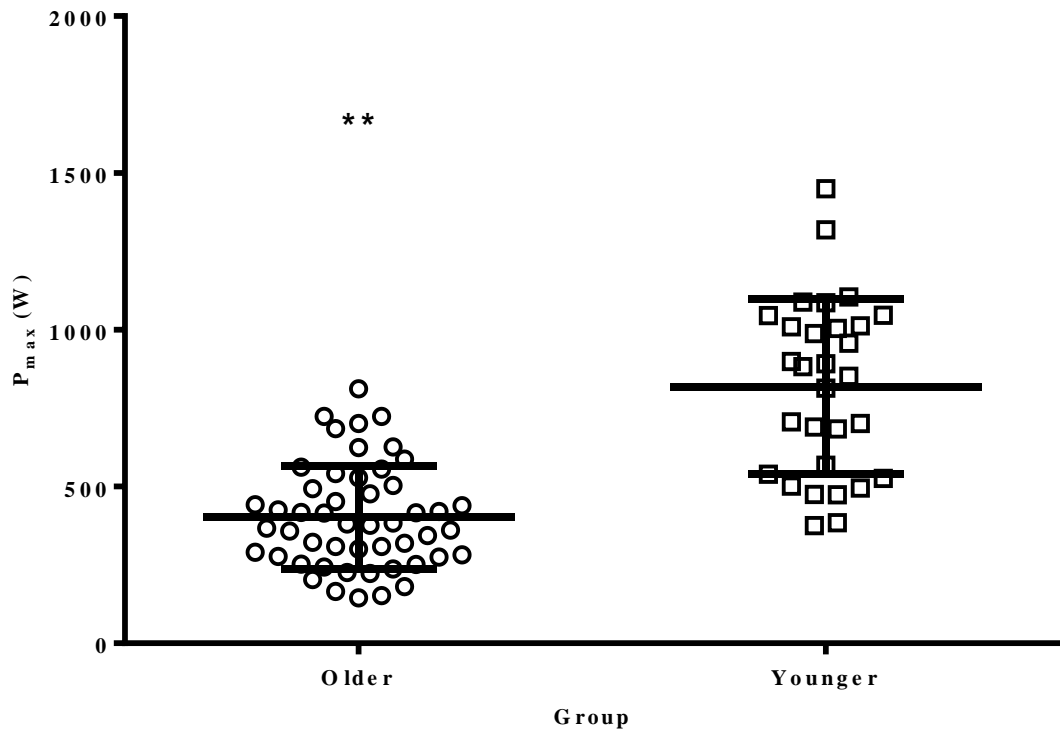


Figure 2.8. Mean \pm SD interpolated peak power (P_{max}) for 65-80 vs 20-35 yr olds. **denotes significant difference between groups ($p < 0.01$).

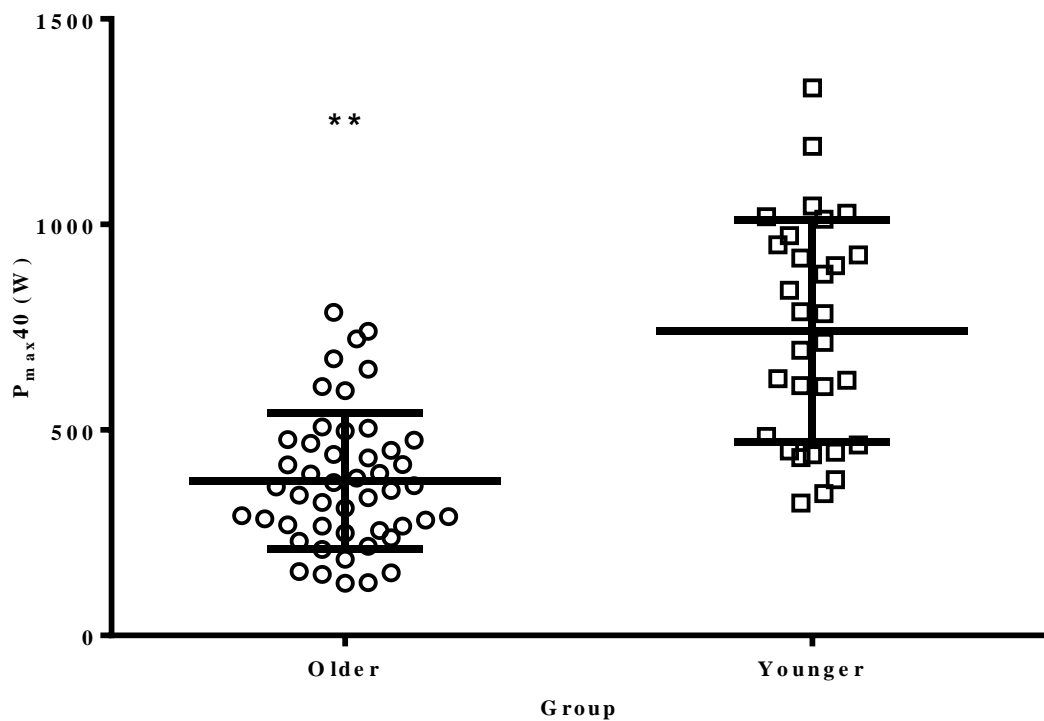


Figure 2.9. Mean \pm SD of mean power from eight discrete repetitions at 40% of one repetition maximum (P_{max40}) for 65-80 vs 20-35 yr olds. **denotes significant difference between groups ($p < 0.01$).

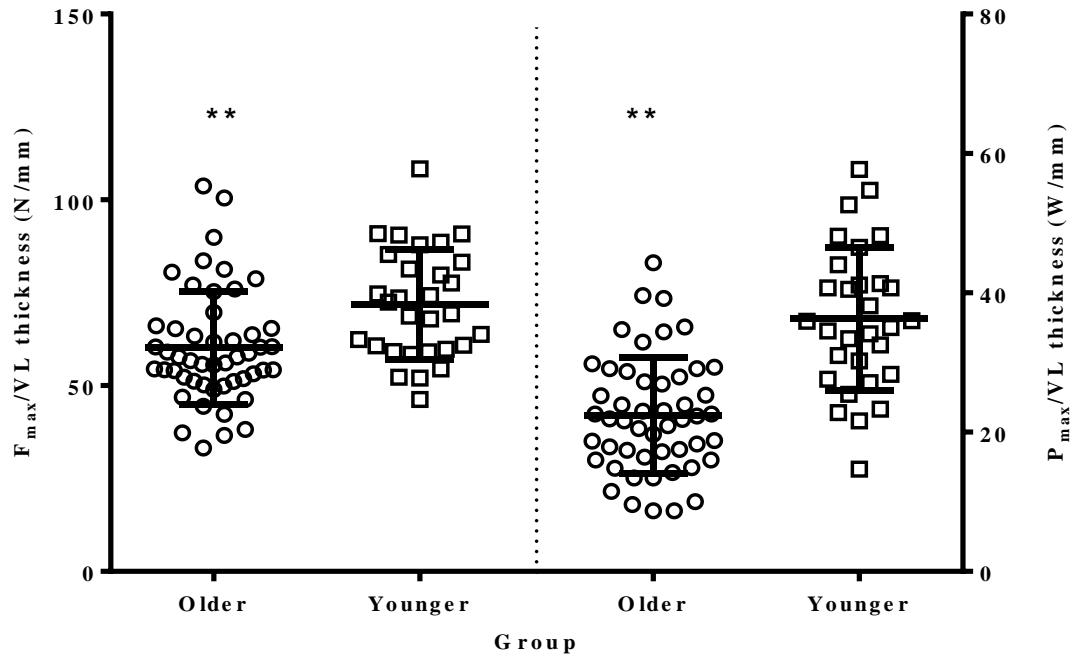


Figure 2.10. Mean \pm SD extrapolated peak force (F_{\max} :VL) and interpolated peak power (P_{\max} :VL) relative to *vastus lateralis* muscle thickness for 65-80 vs 20-35 yr olds. **denotes significant difference between age groups ($p < 0.01$).

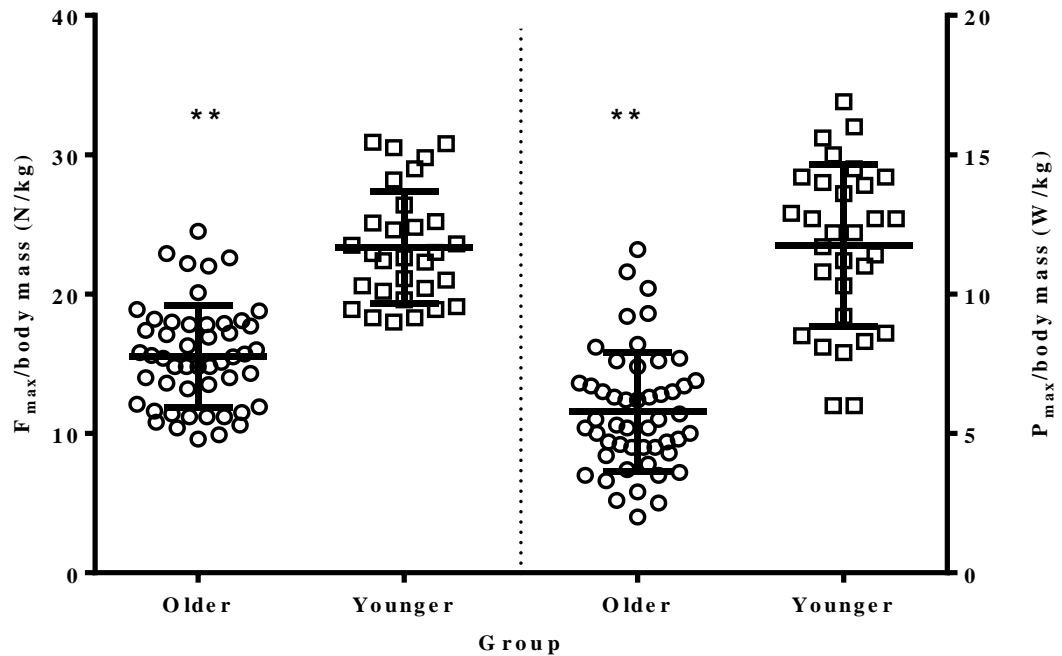


Figure 2.11. Mean \pm SD extrapolated peak force (F_{\max} :kg) and interpolated peak power (P_{\max} :kg) relative to body mass for 65-80 vs 20-35 yr olds. **denotes significant difference between age groups ($p < 0.01$).

2.3.2 Physical activity

Physical activity data for two female participants in the older group could not be obtained due to technical issues, thus participant characteristics for the older group included in the physical activity analyses were: Age 69 (4) years; body weight 69.7 (12.5) kg; BMI 24.4 (3.4) kg/m²; VL thickness 18.2 (4.0) mm; and SPPB score 11 (1) /12.

Physical activity level (PAL) was significantly lower in older compared to younger individuals (1.59 (0.17) vs. 1.74 (0.23), $p < 0.01$, $g = 0.77$). Using age specific thresholds for classifying physical activity based on METs, minutes per day spent being sedentary were not different between the older and younger groups (1058 (112) min/d vs. 1017 (128) min/d, $p = 0.14$, $g = 0.35$). Older individuals spent more time engaging in MVPA than those in the younger group (103 (49) min/d vs. 49 (29) min/d, $p < 0.01$, $g = 1.27$), when using age specific physical activity thresholds.

Muscle function (V_{\max} , F_{\max} , P_{\max} , and $P_{\max40}$) and PAL were not associated in either the older group or younger group (see Figures 2.12-2.15). Muscle function and minutes per day spent sedentary or engaging in MVPA were not significantly correlated in either group.

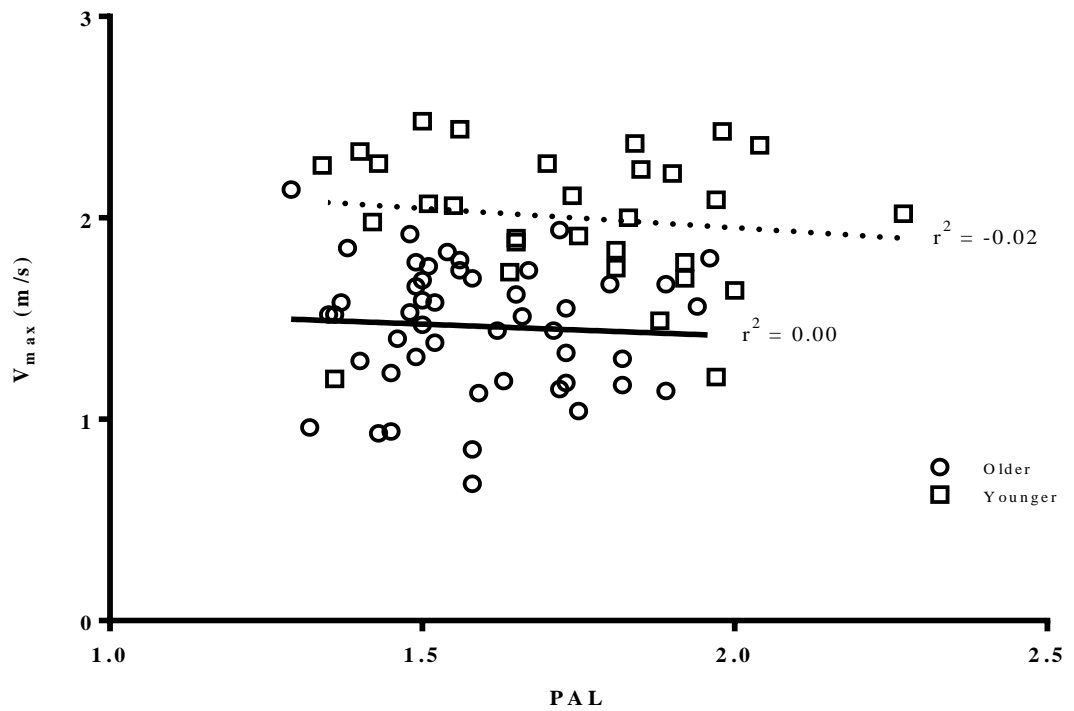


Figure 2.12. Pearson's correlations between daily physical activity level (PAL) (ratio of total energy expenditure to basal metabolic rate) to extrapolated maximum velocity (V_{max}) for 65-80 and 20-35 year olds.

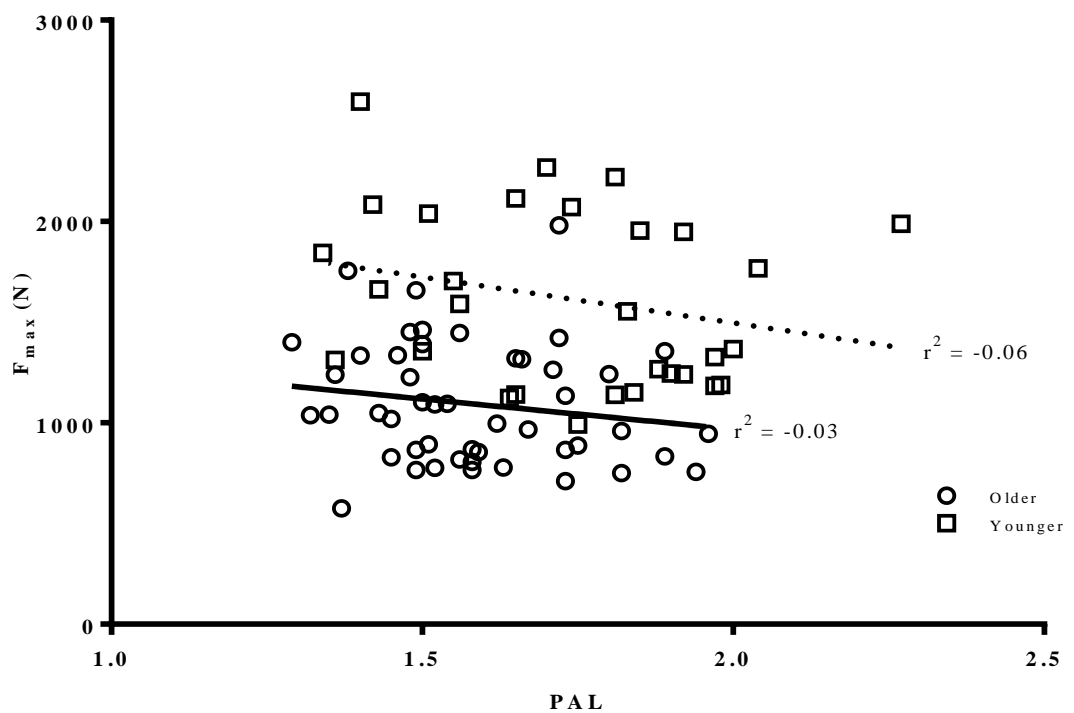


Figure 2.13. Pearson's correlations between daily physical activity level (PAL) (ratio of total energy expenditure to basal metabolic rate) to extrapolated maximum force (F_{max}) for 65-80 and 20-35 year olds.

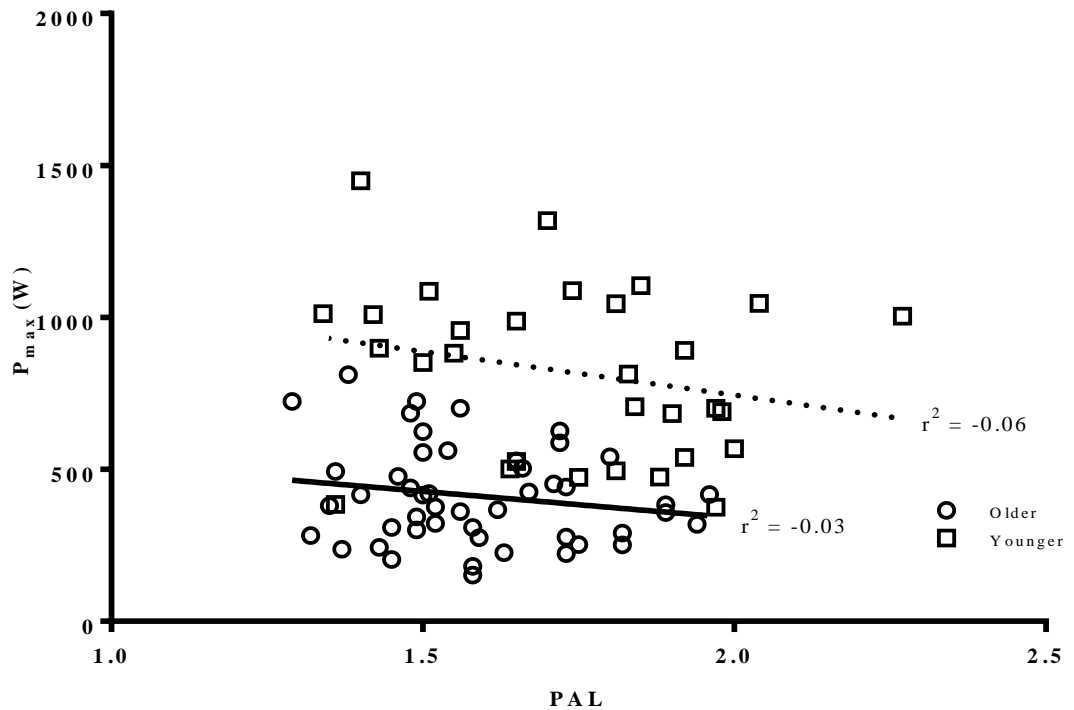


Figure 2.14. Pearson's correlations between daily physical activity level (PAL) (ratio of total energy expenditure to basal metabolic rate) to interpolated maximum power (P_{max}) for 65-80 and 20-35 year olds.

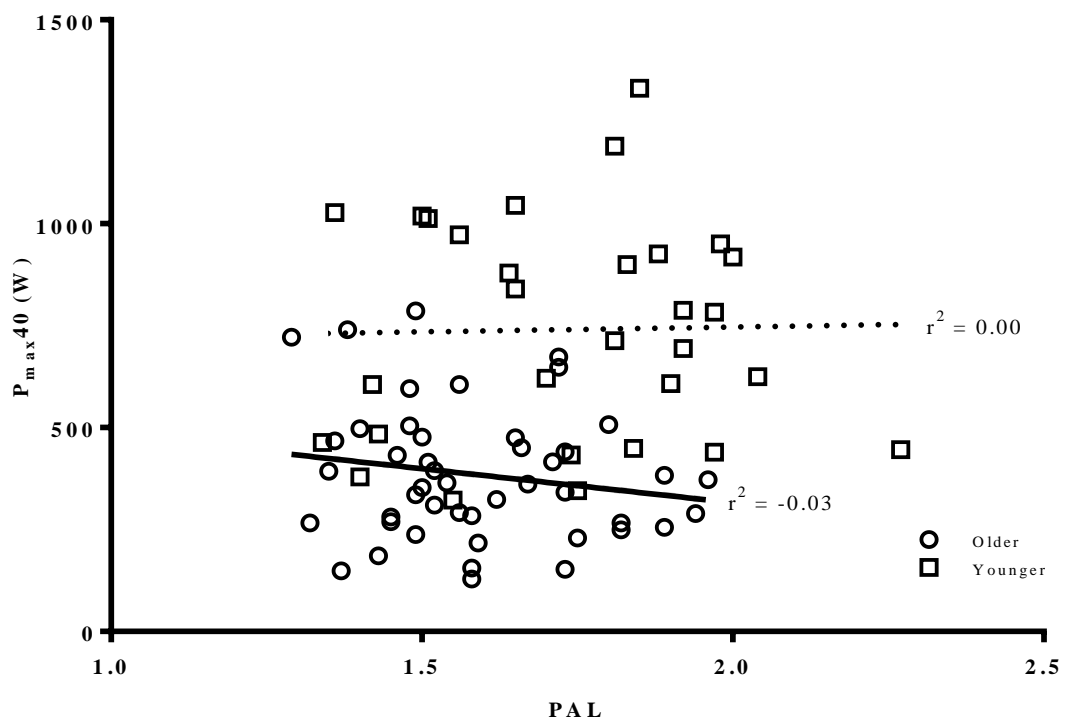


Figure 2.15. Pearson's correlations between daily physical activity level (PAL) (ratio of total energy expenditure to basal metabolic rate) to of mean power from eight discrete repetitions at 40% of one repetition maximum (P_{max40}) for 65-80 and 20-35 year olds.

2.4 Discussion

The main finding was that maximum movement velocity and force at peak power during leg pressing were lower in older individuals compared to younger individuals, resulting in markedly lower peak power. Peak power at 40% of 1-RM was also markedly lower in older compared to younger individuals. Older individuals had lower VL muscle thickness, but force and power producing capability were also significantly lower per unit of muscle thickness in older compared to younger individuals. Overall physical activity level (PAL) was lower in the older group than the younger group (1.59 (0.17) vs. 1.74 (0.23) respectively), however the older group undertook more daily MVPA than the younger group when applying age-specific cut-offs. Despite the older group being reasonably physically active, neither PAL nor MVPA were associated with muscle function in either group.

It has long been recognised that force producing capabilities of muscle decline with age, and that muscle power is lost at a faster rate than strength (Skelton *et al.* 1994). The present data support this; whilst F_{\max} was 33% lower in the older compared to the younger group, P_{\max} was 51% lower. When assessing power with repeated measurement at what should be approximately maximum (Frontera and Ochala 2015), i.e. without the need for interpolation, $P_{\max 40}$ was also 49% lower in the older group. *Vastus lateralis* muscle thickness was 19% lower in the older compared to the younger group and therefore lower force and power production would be expected (Fukunaga *et al.* 2001). However, using force or power per unit of VL muscle thickness as an approximation of specific force, F_{\max} was still lower by 16% and P_{\max} by 38% in the older group. Given that there was no difference in body mass between age groups, that F_{\max}/kg was 33% lower, and P_{\max}/kg was 51% lower demonstrates the overall compromised functional capacity for carrying out day-to-day weight bearing tasks that is apparent with age associated loss of strength and power. Potential causes of reduced force and power producing capabilities relative to muscle size with ageing include decline in neuromuscular activation (Clark *et al.* 2013), architectural changes such as changes in tendon stiffness (Kubo *et al.* 2007) and fat infiltration (Delmonico *et al.* 2009), and possibly deterioration of specific force at the single fibre level (D'Antona *et al.* 2007). Irrespective of the mechanism, deteriorating muscle power in older age represents a cause for concern. In an analysis of factors related to functional status in older women, Foldvari *et al.* (2000) identified leg muscle power as the strongest

univariate correlate of functional status, above maximum oxygen uptake capacity, muscle strength, physical activity levels, and even age. Furthermore, leg muscle strength has been demonstrated to predict mortality over six years of follow-up in men and women over the age of 70, despite no association between mortality and muscle mass (Newman *et al.* 2006).

The older group in the present study represented a well-functioning (SPPB score of 11 (1)) and reasonably active population (PAL = 1.59 (0.17)) (Brooks *et al.* 2004). In ‘absolute’ terms, physical activity accounted for proportionally less of the daily TEE in the older group, however the older group performed more than twice the daily MVPA relative to the young group. Despite this, habitual physical activity levels did not correlate with muscle function, either in absolute terms (F_{\max} and P_{\max}) or when considered per unit of VL muscle thickness (data not shown). Notwithstanding the intuitive appeal of physical activity being positively associated with muscle strength into older age, self-reported physical activity had no association to maximal leg muscle strength in 850 elderly adults, although it was strongly predictive of general motor function (Buchman *et al.* 2007). Furthermore, it would seem that even declining physical activity longitudinally assessed over 10-years by questionnaire does not directly explain declines in strength in relatively active older adults (Hughes *et al.* 2001). The present findings demonstrate that even accurate and objectively measured PAL or time spent engaging in MVPA are not associated with maximal muscle function capabilities in healthy, active >65 year olds.

It is not clear why habitual physical activity was not predictive of muscle function characteristics. It may be that the specific movements in everyday life that could be important for muscle strength or power do not contribute to overall physical activity energy expenditure. Indeed, whilst the Actiheart™ device is an accurate and reliable measure for assessing habitual ambulatory physical activity (Brage *et al.* 2005), these devices cannot capture ‘natural’ resistance/loading exercise such as stair climbing or hill walking with additional load of shopping bags for instance. Moreover, it may be that relatively well-functioning older individuals such as those in the present study may be above a threshold at which habitual physical activity impacts muscle function characteristics directly (Hughes *et al.* 2001). In any case it should be emphasised that the present data does not undermine the wealth of evidence for the importance of maintaining a physically active lifestyle into older age (Haskell *et al.* 2007). In a more

heterogeneous sample of older adults the variability in muscle function may be associated with habitual physical activity level (Jantunen *et al.* 2017). Indeed, the dramatic impact on muscle function and mass of removing, or even just reducing, daily activity for a short period of time explains this (Kortebein *et al.* 2008; Breen *et al.* 2013). The present data do highlight that lower limb muscle function in terms of maximal velocity, force, and power producing capabilities, is impaired in older individuals compared with younger individuals even when remaining active, and focus needs to be placed on recognised strategies to maintain muscle function such as resistance exercise (Liu and Latham 2009; Peterson *et al.* 2010). Moreover, greater strength and power are themselves associated with improved health outcomes in later life, and essentially facilitate that tasks of daily living are in relative terms less strenuous, thus likely delaying onset of frailty (Reid and Fielding 2012).

There are limitations to consider in the present findings: for example a measure of all leg extensor muscle volume would have been a more accurate basis for assessing muscle force producing capability relative to size than ultrasound measurement of VL alone (Fukunaga *et al.* 2001). No measures of motor unit recruitment efficacy (for example twitch interpolation) or muscle fibre type characteristics were recorded, which would have provided insight into the mechanism by which force and power producing capability degenerate with ageing (Gandevia 2001; Yu *et al.* 2007). Additionally, whilst outcomes such as muscle function remain relatively stable, with physical activity levels being inherently malleable the Hawthorne effect may have impacted activity patterns, despite participants being instructed to maintain their regular lifestyle (Bravata *et al.* 2007). Whether this would have differed between groups cannot be estimated. Furthermore, the potential for seasonal variation in activity and indeed the lifetime history of physical activity should be considered in future work (Zampieri *et al.* 2014).

In conclusion, velocity and force producing capability of muscle decreases with age even in active older adults, which translates to a reduction in muscle power production both in absolute terms and relative to muscle size. However, the variability in muscle function characteristics during dynamic leg pressing was not explained by objectively measured habitual physical activity. As such, whilst total physical level is important for many indices of healthy ageing, targeted efforts, such as resistance exercise, are likely a requirement to maintain muscle function into later life.

Chapter 3: Exercise snacking to improve muscle function in healthy older adults- A pilot study

3.1 Introduction

Frailty is underpinned by a progressive loss of muscle mass and strength, particularly from the lower limbs, and is associated with increased risk of falls and reduced quality of life (Faulkner *et al.* 2007; Marcell 2003). There exists a minimum threshold of strength and function required to complete tasks of daily living independently. Recognising means to delay individuals reaching this ‘frailty threshold’ has been identified as an urgent health care priority (Cooper *et al.* 2012). Crucially, action is needed before older adults reach a point where it is not possible to safely exercise to recover lost muscle strength. With muscle mass lost at 0.5-1% per year after 50 years of age (Mitchell *et al.* 2012), even modest improvements of a few percent in muscle size and strength from a training programme may essentially represent postponement of frailty measurable in years.

Progressive resistance exercise training is an efficacious means to improve muscle strength in older adults, and is accompanied by multifaceted improvements in health and function (Liu and Latham 2009; Frank *et al.* 2015). Traditionally, heavy load resistance training has been regarded as the most effective strategy to increase muscle strength, due to associated neural and hypertrophic adaptations (Schoenfeld *et al.* 2016a; Folland and Williams 2007a). Recent evidence suggests low load resistance training can also be efficacious in increasing muscle strength, particularly in an untrained population, albeit to a moderated degree in comparison to high load resistance training (Morton *et al.* 2016; Schoenfeld *et al.* 2016b).

It has been suggested that in already trained individuals, manipulating training frequency to maintain overall training volume across a week with more sessions of lower load may in fact increase hypertrophy (Dankel *et al.* 2016). Whilst this hasn’t yet been borne out empirically (Schoenfeld *et al.* 2016a), it is intuitively appealing to reason that a reduced training session load with short recovery times, which still induces some hypertrophic stimuli, would suit an older population previously doing no exercise. In this respect, a model of daily body weight exercise could be suitable for undertaking in the home without the need for supervision.

A key contributor to sarcopenia is anabolic resistance, i.e. symptomatically reduced MPS response to amino acid feeding, resulting in a negative mismatch between overall protein synthesis and degradation (Burd *et al.* 2012). However, this is a somewhat plastic state that can be overcome by manipulating diet and performing resistance exercise, thus representing a crucial target to stem progression of sarcopenia (Moore 2014; Symons *et al.* 2011; Dideriksen *et al.* 2013). Agergaard *et al.* (2017) demonstrated that high volume, low load resistance training robustly increased MPS response to protein feeding for at least seven hours post-exercise in men over 65 years of age. Moreover, simply increasing the protein content of a meal may also help overcome the challenge of anabolic resistance (Paddon-Jones *et al.* 2015). Adding protein to meals that traditionally aren't protein rich, such as breakfast, may therefore be a pragmatic means of increasing both per meal protein content and overall protein content of the diet for older adults (Cardon-Thomas *et al.* 2017). Additional dietary protein may optimise the response to low load resistance training (Atherton and Smith 2012).

The aim of the present study was to investigate the effects of four weeks of twice daily 'exercise snacking' on maximum number of sit-to-stands from a chair performed in one minute, compared to a control group continuing their habitual physical activity patterns. To support the potential anabolic effects of the exercise snacking regime, both the control group and exercise snacking group received 16.5 g of extra daily protein at breakfast. Thereafter, the impact of exercise snacking on static balance and ambulatory capacity, lower limb muscle and neuromuscular function during dynamic leg pressing and isometric plantar flexion respectively, and whole body and lower limb anthropometry were explored. It was hypothesised that exercise snacking for 28 days would increase the number of sit-to-stands performed in one minute. The effects of the exercise snacking intervention on static balance and ambulatory capacity, lower limb muscle and neuromuscular function during dynamic leg pressing and isometric plantar flexion respectively, and whole body and lower limb anthropometry were expected to be limited based on the nature of the intervention.

3.2 Methods

3.2.1 Participants

Twenty healthy, community dwelling, older men and women (65-80 years), not undertaking regular structured exercise, were recruited for the pilot study through local newspaper advertisement and social media. Potential participants were fully informed as to the study design and methodology with written information, and an informal telephone interview was conducted by a member of the research team to ascertain initial eligibility, provide a verbal overview of the study, and discuss the feasibility of that individual's participation. Individuals who were non-smokers, BMI $\geq 20 < 30$ kg/m², had no contraindications to exercise or recent history of musculoskeletal injury, and scored 8 or above with no score of zero on any test of the SPPB (Guralnik *et al.* 1994) at the initial screening were invited to take part in the pilot study. During the screening visits, BMI was calculated from height and weight measured with a wall mounted stadiometer (222 Seca, Hamburg, Germany) and balance scales (424 Weylux, H Fereday & Sons Ltd, London, UK) respectively. All participants provided written informed consent. The protocol was approved by the NHS South West – Frenchay Research Ethics Committee (REC) (Ref: 16/SW/0300). Participant characteristics are presented in Table 3.1.

Table 3.1. Participant characteristics

	Control ($n = 10$; ♀ = 7)	ES ($n = 10$; ♀ = 7)
Age, years	74 (5)	70 (4)
Body mass, kg	70.9 (11.9)	69.7 (9.9)
BMI, kg/m ²	26.3 (3.5)	25.0 (3.4)
SPPB score	11 (1)	12 (1)
STS score at screening	29 (12)	29 (10)
PAL at screening	1.63 (0.19)	1.70 (0.14)

Data presented as mean (standard deviation). ES: Exercise-snacking group, BMI: Body mass index, SPPB: Short physical performance battery, STS: 60 second sit to stand test, PAL: Physical activity level (ratio of total energy expenditure to basal metabolic rate)

3.2.2 Procedures

Participants visited the laboratory on four occasions; for an initial screening and familiarisation with function measures, a second familiarisation session, and pre- and post-intervention trials. All participants underwent seven days of baseline physical activity monitoring assessed by a wearable physical activity monitor (BodyMedia armband), and completed a three-day food diary, following the initial screening visit. See Figure 3.1 for a schematic overview of the study protocol.

On the day before and day after the 28-day intervention, participants were asked to arrive at the laboratory for trial days following a 10 hour overnight fast, having drunk 1 pint of water, and having not undertaken exhaustive exercise in the previous 24 hours. Imaging measures were undertaken, and participants were then provided with a breakfast of their choice which was matched on the second visit. Functional measures of balance, six-minute-walk-test (6MWT), 60 second sit-to-stand test (STS) from a chair, leg press dynamometry (A420, Keiser®, Fresno, CA), and plantar flexion twitch interpolation testing, were then undertaken. Apart from plantar flexion twitch interpolation, all tests were performed shod, with footwear and clothing worn matched on pre- and post-intervention trials.

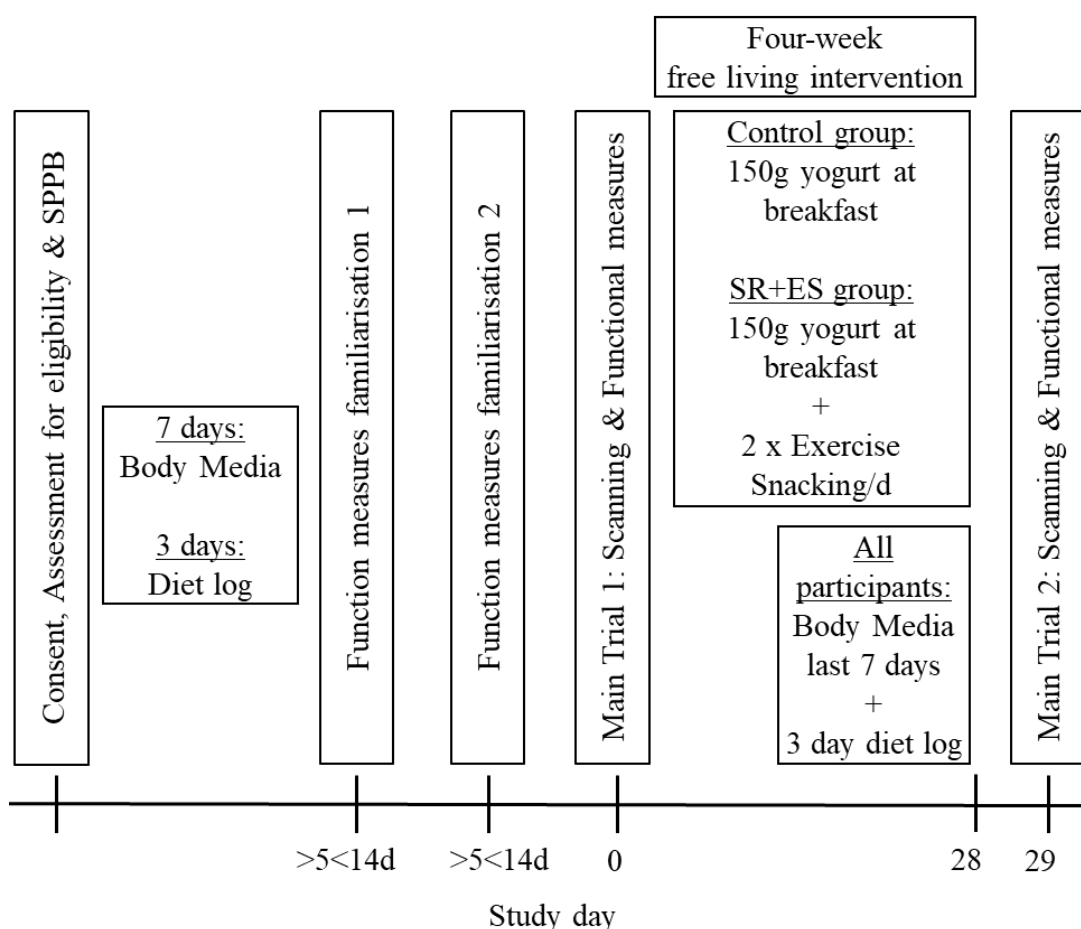


Figure 3.1. Schematic overview of the study timeline (d = day; SPPB = Short physical performance battery)

3.2.3 Intervention

Participants were assigned to groups by way of minimisation to limit differences in mean age, BMI, and STS score at the screening visit, on account of the small sample size (Altman and Bland 2005). The control group were asked to continue with their habitual physical activities, whilst consuming 150 g of yogurt (Natural flavour, Skyr, Arla®; 98 kcal, 0.3 g fat, 6 g carbohydrate, 16.5 g protein) for 28 days as part of their breakfast meal. Participants were provided with a log book to record whether the yogurt had been consumed each day, and deliberately not given any further instruction with regards to dietary intake. The exercise snacking group were asked to consume 150 g of yogurt as part of their breakfast meal, and perform two bouts of ‘exercise snacking’ per day, once in the morning and once in the evening. Each bout of exercise snacking included five exercises, each undertaken for one minute with the aim to complete as many repetitions as possible in that minute. Between each exercise, participants rested for 60 seconds. The exercises were: sit-to-

stand from a chair (Figure 3.2), standing alternating knee bends (Figure 3.3), marching on the spot (Figure 3.4), seated alternating knee extension (Figure 3.5), and standing calf raises (Figure 3.6). Participants were advised to hold onto a chair for stability during standing exercise if they felt the need to. The sit to stand exercise was performed first, with the number of repetitions completed recorded in a provided log book as a means to assess adherence. During the last seven days of the intervention, both groups wore a physical activity monitor (SenseWear, BodyMedia, Inc., Pittsburg, PA, USA), and completed a three-day food diary.

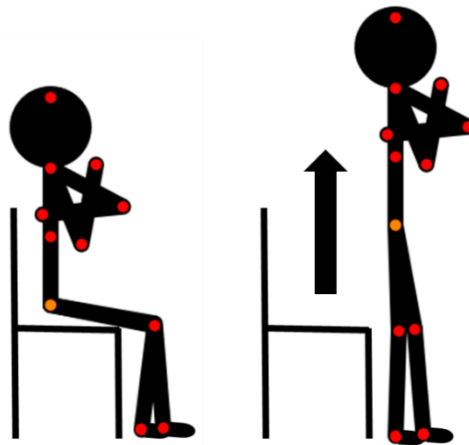


Figure 3.2. The sit-to-stand exercise- participants crossed their arms to prevent them using them for assistance, and repeatedly rose from a seated position in a chair to fully standing.

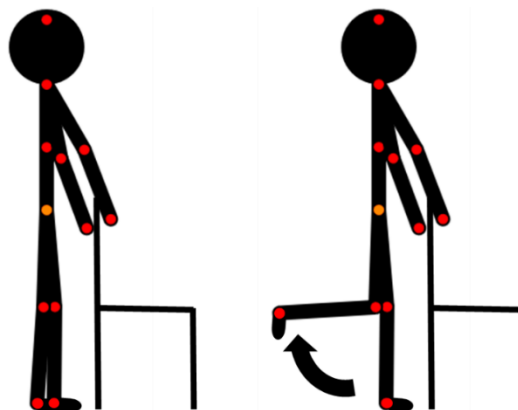


Figure 3.3. The standing alternating knee bend exercise- participants held the back of a chair for balance if needed, and repeatedly bent one knee at a time to the point where their shank was roughly parallel to the floor, alternating legs for each repetition.

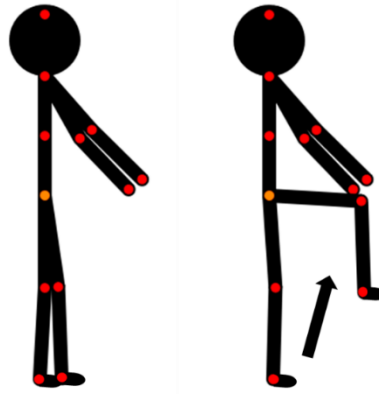


Figure 3.4. The march on the spot exercise- participants held their arms out in front of them for balance, with hands at roughly waste height, then marched on the spot such that their thigh was roughly parallel with the floor at the top of the movement range.

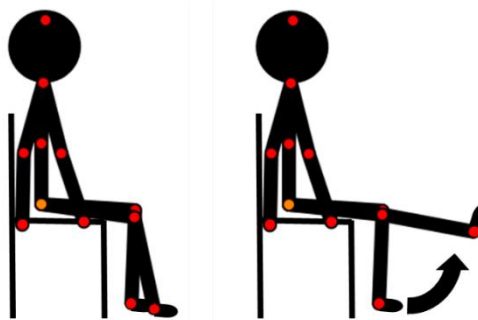


Figure 3.5. The seated alternating knee extension exercise- participants sat in a chair, and repeated extended one knee at a time to the point that their knee was near to full extension at the end of the movement range, alternating legs for each repetition.

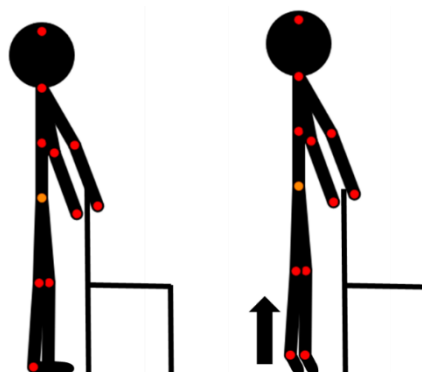


Figure 3.6. The standing calf raise exercise- participants held on to the back of a chair for balance, starting from a flat footed stance with weight equally distributed on both feet, they repeatedly rose up onto their toes as high as they could without losing balance.

3.2.4 Functional measures

Maximum number of repeated STS in 60 seconds were performed from a chair with a seat height of 44 cm, with arms folded across the chest, and reaching full hip and knee extension on standing. During familiarisation, a researcher counted the number of repetitions aloud, with a timing clock in view of the participant. On trial days, participants were not in view of a clock and repetitions were not counted aloud, with participants instructed to complete repetitions at the fastest rate they could manage until told to stop.

The 6MWT was conducted with a 10-m walkway, demarcated with cones placed 20 cm back from each end around which participants turned anti-clockwise. Participants were instructed to walk at a self-selected speed that was comfortable, but to aim to cover the maximum distance possible in 6 minutes. Investigators did not walk with the participant, and no encouragement, indication of distance covered, or time elapsed, were provided during the test. Rating of perceived exertion (RPE) was assessed using Borg's RPE 15-grade scale (Borg and Dahlstrom 1962) for both the STS and 6MWT immediately on completion of each test.

Postural sway (centre of pressure (CoP) mean speed, and anterior-posterior displacement range (AP range)) were assessed initially with six trials of 40 seconds quiet standing on a force-plate (9287BA, Kistler Instruments Ltd., Switzerland) performed with feet approximately 15 cm apart in a comfortable position, three with eyes open and three with eyes closed, with trial condition order randomised. A further two trials of 60 seconds standing balance were performed with feet together and heels touching, one with eyes open, the other with eyes closed, in a randomised order. Ground reaction force (GRF), CoP, and free movement data were collected and processed at 500 Hz using Qualisys Track Manager (v2.14, Qualisys, Gothenburg, Sweden). Data were re-sampled in Visual 3D (v6.01.5, C-Motion Inc., Maryland, USA) to 100 Hz and filtered with a Butterworth low pass filter with a cut off frequency of 5 Hz, and exported for later analysis using Microsoft® Excel® 2013. Trials performed with the eyes closed were ended if the participant lost balance or stepped out of the respective quiet standing position, opened their eyes, or felt that they could not balance. An investigator stood behind the participant but out of eye line in case of loss of balance.

Lower limb velocity, force, and power production characteristics, was measured on a seated pneumatic leg press dynamometer (A420, Keiser®, Fresno, CA) using the incremental power test and 8-rep test at 40%-1RM as per methods described in Chapter 2. On trial days a set warm-up based on the 1-RM achieved in the second familiarisation was performed, consisting of 5 x 30%-1RM, 5 x 50%-1RM, 2 x 70%-1RM, 1 x 80%-1RM. Five minutes of seated rest was observed between the incremental power test and eight repetition tests. Data were processed and analysed as per methods described in Chapter 2. As per Chapter 2, the eight repetition test was included to examine whether any changes seen in leg pressing strength or power were consistently observed across multiple repetitions of the same resistance, rather than single repetitions at incremental resistances as used in the incremental power test. Again, 40% of 1-RM with 60 seconds rest was used to ensure that there was no risk that the measurement would be impacted by fatigue.

Plantar flexion isometric maximal voluntary force (MVF) and interpolated twitch ratio (ITR) were measured from the dominant leg with the ankle dorsiflexed at 10° , using the apparatus developed by Davies *et al.* (1982). Participants were seated upright in a custom-made rig (see Figure 3.7), with hips at 90° and arms folded across the chest to prevent participants pulling or bracing on the rig.



Figure 3.7. Image of the custom made rig that was used to measure isometric plantar flexion and conduct the twitch interpolation testing. Participants were seated on the wooden bench with hips at 90° and back upright, with arms were folded across the chest. The foot of the dominant leg was placed on the white plate, with additional plates added to raise the foot such that thigh was horizontal when the ankle was 10° dorsiflexed, with the knee was placed in the brace.

The knee was held under a rigid cuff fixed to a sprung steel bar instrumented with a custom-made force transducer of a strain gauges (CEA-06, Micro Measurements, Vishay Precision Group, Inc., Malvern, Pennsylvania, USA) in a full Wheatstone bridge force formation (see Figure 3.8). The force transducer was calibrated with a load cell and amplifier (9331B Quartz Force Link, and 5011B10 Charge Amplifier respectively, both Kistler, Winterhur, Switzerland).

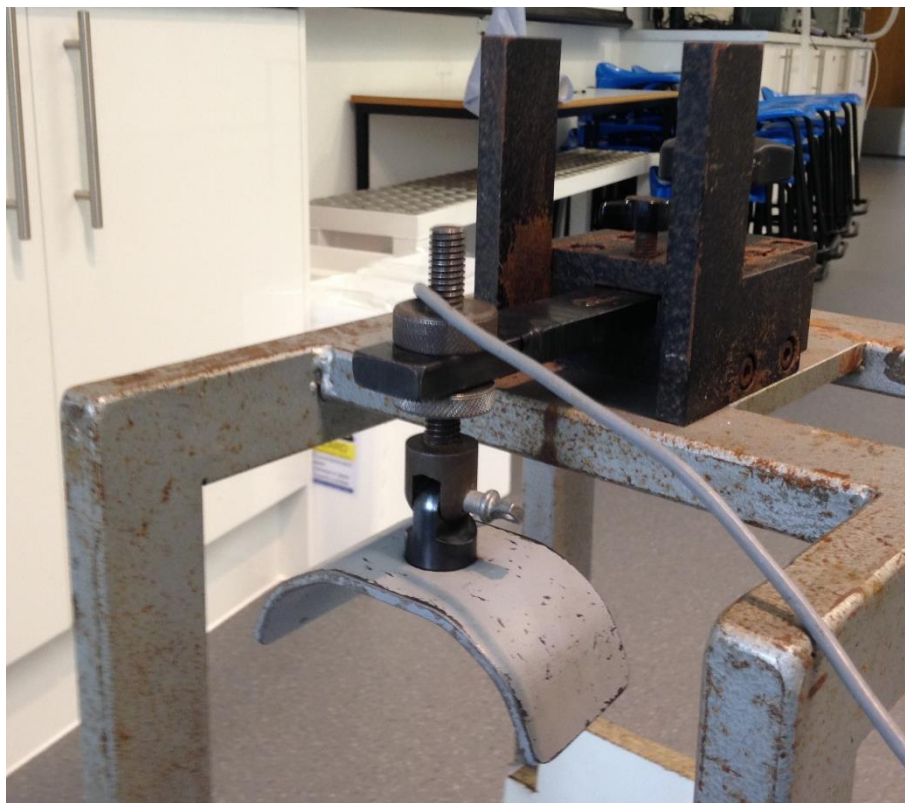


Figure 3.8. The knee brace attached to the sprung steel bar, instrumented with a strain gauge (not shown). No padding was added to the knee brace to prevent dissipation of force during plantar flexion, thus the position of the knee was such at the distal edge of the knee brace was slightly proximal to the femoral epicondyles.

Constant-voltage electrical stimulation of the plantar flexors was delivered percutaneously (5 x 9 cm rectangle ValuTrode Cloth, Axelgaard Manufacturing Co. Ltd, Fallbrook, California, USA) with an electrical stimulator (DS7-AH, Digitimer Ltd, Welwyn Garden City, UK). The anode was placed immediately distal to the popliteal crease, with the cathode over the distal myotendinous junction of the soleus. Force and electrical stimulation timing data were collected at 500 Hz (cRIO-9024 programmed in Labview v2010, National Instruments, Austin, Texas, USA). Custom-made functions were written in Matlab (R2015b, TheMathWorks Inc., USA) to process data and to extract the variables of interest from each trial. Participants performed three 3 second MVC plantar flexions with two minutes rest between efforts, with strong verbal encouragement from the investigators. Thereafter, whilst participants maintained a relaxed muscle, stimulation single pulses of 50 μ s with increasing amperage were applied until no further twitch force was obtained (see Figure 3.9).

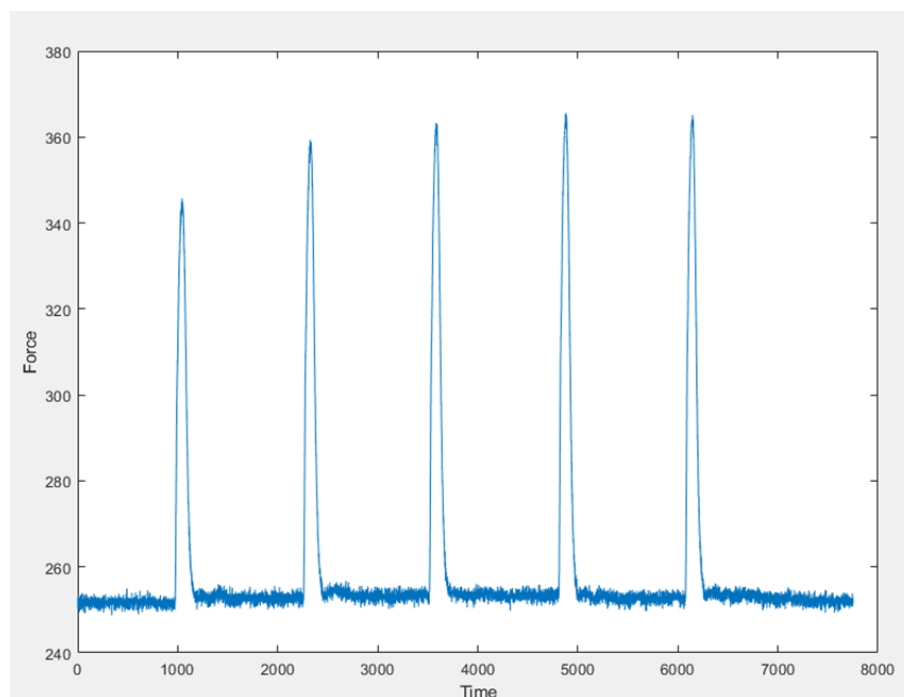


Figure 3.9. An example of a typical force trace obtained by incrementally increasing single pulse stimulation by 50 μ s until no figure force output was observed, to establish the amperage needed to achieve maximal twitch force whilst at rest.

This current was increased by 50 mA, and was used as the supramaximal stimulation for the twitch interpolation trials. After three minutes of rest, participants performed three 3 second MVC's with evoked twitches, with two minutes rest. Supramaximal stimulation was applied at the point of maximum voluntary force production of the contraction based on real time feedback from the data collection software (see Figure 3.10). Participants were instructed to cease contraction immediately after the twitch, and within three seconds a potentiated control twitch was delivered (Tc).

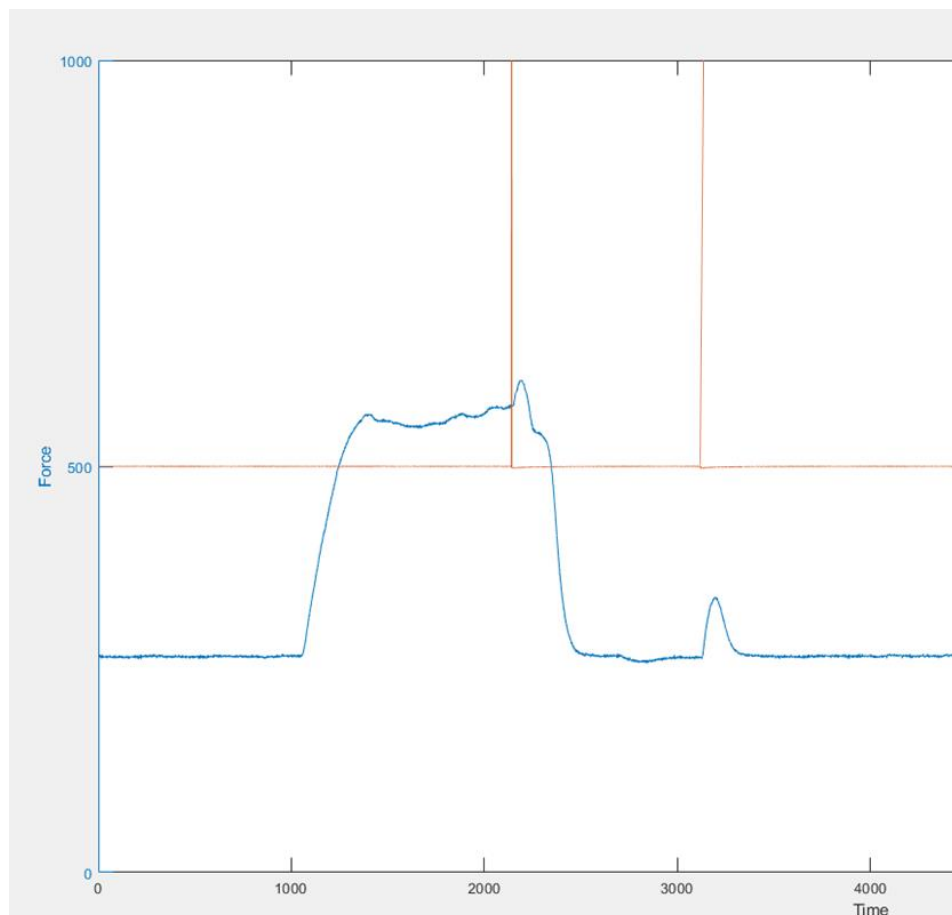


Figure 3.10. An example force trace from an assessment of neural drive using the interpolated twitch technique for plantar flexion. The force output is represented by the blue line, and the neural stimulation by the orange line from a secondary y-axis not shown. The first twitch was delivered when the isometric contraction reached a force corresponding to that previously achieved in maximal voluntary contractions. A second neural stimulation was delivered with three seconds of the participant relaxing, whilst the muscle was in a potentiated state. The force generated in the twitch response during contraction was considered indicative of the proportion of voluntarily activated motor units, when compared to the force generated by the potentiated twitch.

Data were reported from the trial with the greatest voluntary force at instant of evoked twitch, and trials in which participants achieved <85% of their MVF were discarded.

Interpolated twitch ratio was calculated as:

$$\% \text{ muscle activation} = 100 - (\text{force of evoked twitch (Ts)/Tc}) * 100$$

3.2.5 Anthropometry

Weight was measured with electronic scales (BC543, Tanita, Amsterdam, Netherlands) following a 10-hour overnight fast. Whole body composition, whole body lean mass, and leg lean mass were estimated using a DXA system (Hologic Discovery W, QDR software version 12.4.2, Bedford, MA) by differentiating the fat, bone, and lean (non-bone non-fat) masses. A spine phantom was used for the quality control scan performed at the start of every trial day prior to participant testing, as per the manufacturer's guidelines. Participants wore the same light clothes for pre- and post-intervention trials, and removed all metal items. The investigator positioned the participant to be laying supine on the scanning bed such that body regions could be partitioned upon analysis. During analysis, manual placement of boundaries between discrete anatomical regions was conducted for all scans by the same investigator (OJP), before analysis using manufacturer's software. See Figure 3.11 for example image of DXA scan with sub-regions partitioned for analysis.

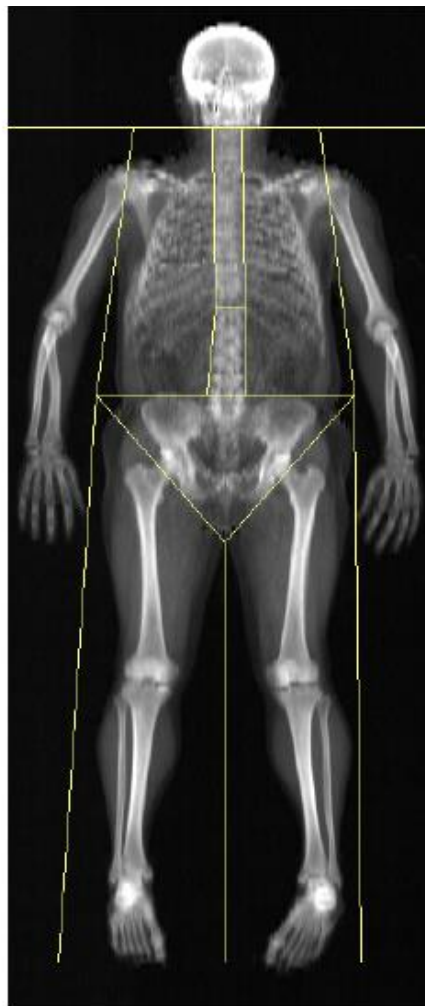


Figure 3.11. Example image of a DXA scan with sub-regions partitioned for analysis.

Lower limb (calf and thigh) mCSA, fat CSA, and muscle density (as a proxy for fat infiltration to the muscle), were assessed by pQCT (XCT3000, StraTec Medizintechnik GmbH, Pforzheim, Germany). During the pre-intervention trial, dominant leg tibia length was measured with a fabric tape measure from medial knee joint line to medial malleolus, and femur length from the great trochanter to lateral knee joint line whilst standing. Scans were performed with the participant laying supine on a bed with leg placed through the scanning gantry and foot strapped into a foot plate. The calf was supported by a custom made pad for thigh scans. Scout scans were performed at the distal ends of the tibia and femur to locate the end of bones respectively. Single 2D slice scans were performed at 66% of the tibia length proximally from the medial malleolus, and 25% of the femur length proximally to the lateral femoral epicondyle, based on the bone lengths previously recorded. Scan images were analysed using the BoneJ plugin (Version 1.4.2) for ImageJ (1.44p, Wayne Rasband, National Institutes of Health, USA) (Rantalainen *et al.* 2013; Doube *et al.* 2010). See Figure 3.12 for example image of analysed pQCT scan with sub-regions partitioned for area quantification by pixel density threshold analysis.

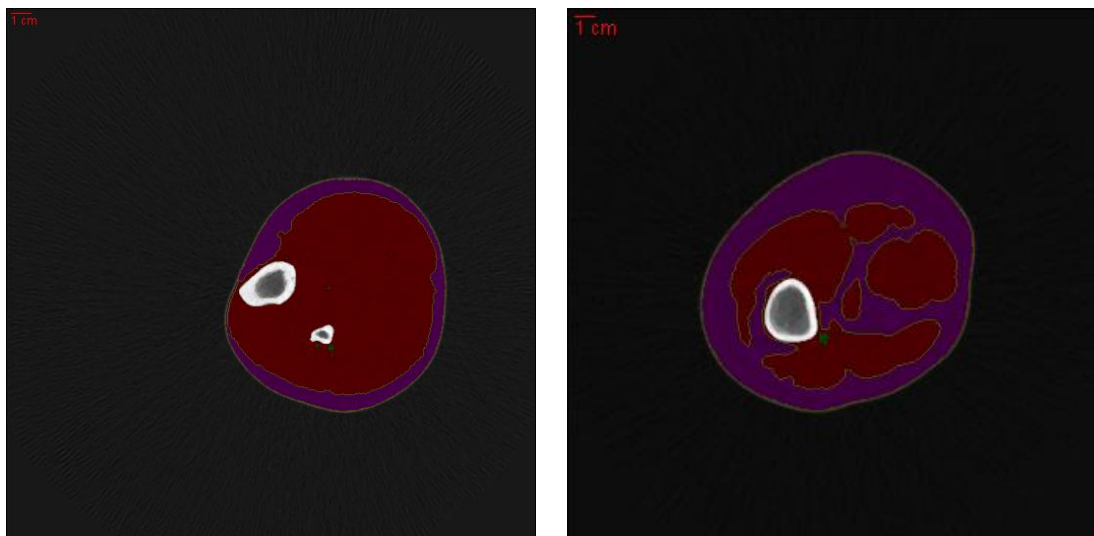


Figure 3.12. Example images of pQCT images after analysis with BoneJ plugin (Version 1.4.2) for ImageJ for 66% tibia site (left), and 25% femur site (right). Bone is represented by white, with bone marrow in grey, and muscle represented in red, with subcutaneous adipose tissue in purple. Intramuscular adipose tissue deposits are represented in green.

The pQCT technique uses the same technical premise of standard computed tomography, in which images are generated based on the linear x-ray absorption coefficients of tissues (muscle, bone, and fat) through which it passes (Adams 2009). The XCT3000 device used in the present study (and Chapter 4), records the x-rays through 12-receivers, and generates a cross-sectional image of a limb as a 2D map of pixels. The technique is becoming more widely used in sarcopenia research given the device portability and negligible exposure to ionizing radiation ($<1 \mu\text{Sv}$ per scan) (Tosato *et al.* 2017; Erlandson *et al.* 2016). The repeatability of the pQCT method for assessing muscle cross-sectional area postmenopausal women was assessed by Frank-Wilson *et al.* (2015). The root-mean-squared coefficient of variation for the repeated measures of images analysed using the BoneJ plugin (Version 1.4.2) for ImageJ, as described above, was 2.5%. This was equivalent to the repeatability when analysis was undertaken with the various Stratec XCT software analysis masks and filters typically used in the literature (2.6%-3.7%). The repeatability of the BoneJ plugin for analysis of muscle density, intramuscular adipose tissue area, and subcutaneous adipose tissue area was significantly better than Stratec XCT software analysis masks. Similarly, Gordon *et al.* (2003) observed a coefficient of variation of 2.5% between two repeated calf muscle scans on the pQCT. Furthermore, the relationship between pQCT measured muscle cross sectional area and traditional spiral CT was $R^2=0.96$ ($p < 0.001$) (Gordon *et al.* 2003).

Estimations of *medial gastrocnemius* (MG) and *vastus lateralis* (VL) muscle fascicle length and pennation angle were taken using ultrasound whilst the participant stood in a comfortable position with weight even distributed onto both feet. Longitudinal images were taken at the widest part of MG, and 50% of thigh length from the dominant leg using a 128 element linear array transducer (LV7.5/60/96Z, TELEMED, Lithuania) operating in B-mode at 8.0 MHz with a scan image depth of 65 mm. All images were processed by the same investigator (OJP) using ImageJ software and custom made functions written in Matlab to extract variables of interest, with each image analysed three times with typical error calculated (Hopkins 2015). The interior border of the superficial and deep aponeuroses, and the most distinguishable muscle fascicle were identified and assumed to be straight (See Figure 3.13). The angle of intersection between the deep aponeurosis and muscle fascicle, and the length between the intersections of the muscle fascicle with the superficial and

deep aponeurosis were defined as the pennation angle and fascicle length, respectively.

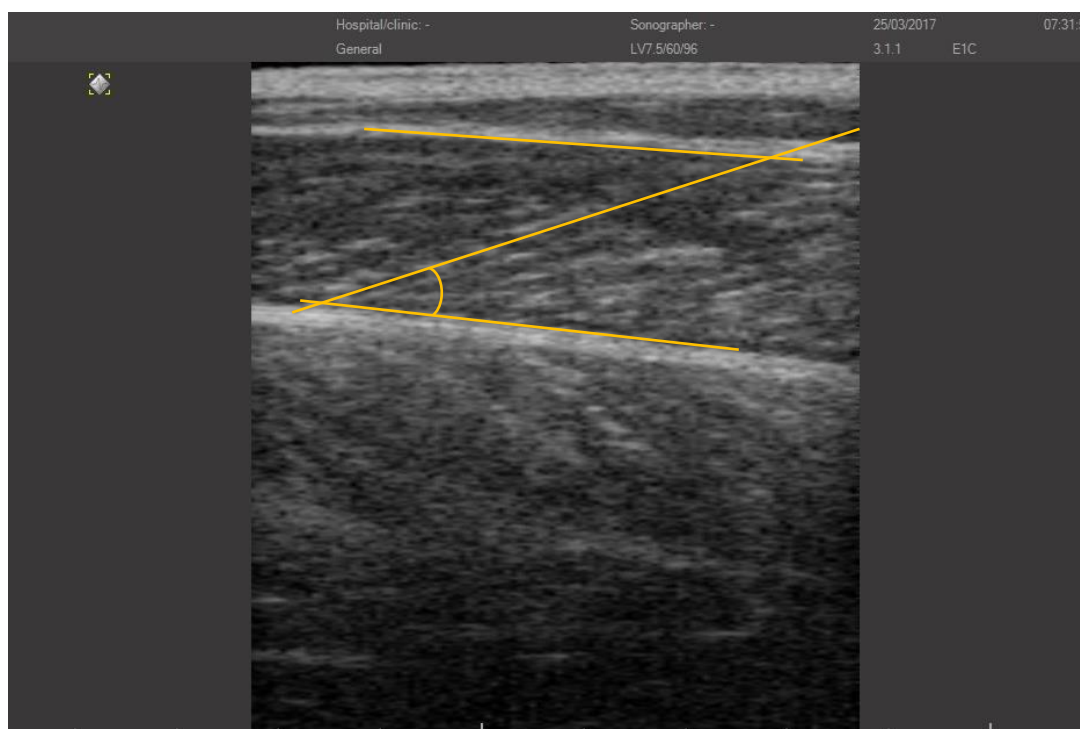


Figure 3.13. Example of an ultrasound scan image of gastrocnemius medialis with lines indicating identification of superficial and deep aponeurosis, and fascicle pennation angle to deep aponeurosis.

3.2.6 Diet records

Three-day diet records (two weekdays, one weekend day) from both baseline, i.e. between screening and pre-intervention trial day, and during the last week of the intervention period, were analysed using Nutritics commercially available online app (v4.312 Nutritics Education, Dublin, Ireland). Mean daily intake of total kcal, and carbohydrates (CHO), proteins (PRO), and fats in grams were obtained, and calculated relative kg body mass using screening body mass and post-intervention body mass for baseline and intervention dietary records respectively.

3.2.7 Physical activity

Physical activity level (PAL) was calculated as estimated mean daily energy expenditure / resting metabolic rate (estimated using the World Health Organisation equation). Only days with >95% wear time achieved were included in the analysis.

Instructed to remove armband for water based activity such as bathing or showering, and any water based sports recorded in the log book.

3.2.8 Sample size and statistical analysis

With the novelty of the intervention, specifically with respect to the frequency and intensity of the exercise, and the study design being without a true control group due to the Control group also receiving a form of intervention (daily yogurt consumption), no quantitative sample size calculation was undertaken. Instead, a pragmatic approach was taken based on the practicalities of the intervention, and the sensitivity of the measures to be employed.

Shapiro-Wilks tests of normality were performed on participant characteristic data recorded at screening (age, body mass, BMI, and STS score) due to the small sample size. Participant characteristic variables were normally distributed, thus compared with independent-samples t-test. Outcome variables were analysed with a two-way repeated measures analysis of variance (ANOVA), and where a significant interaction or time effect was observed, a Holm-Bonferroni *post hoc* test performed. Statistical significance was accepted at $p < 0.05$. To infer the magnitude of differences between groups, Hedges *g* effect size of difference in change scores between groups were calculated, to account for low sample size (Hedges 1982). Effect sizes were classed as small (0.2), moderate (0.5), and large (0.8) according to Cohen (1988). Data are presented as mean (standard deviation), and ANOVA and *post hoc* analysis performed using SPSS v22.0 (SPSS Ins., Chicago, IL), and effect size analysis performed using Microsoft® Excel® 2013.

3.3 Results

3.3.1 Functional measures

Adherence to the ES intervention was 98% (2 (1) sessions out of 56 sessions missed), and no adverse events occurred during the intervention period. Sit-to-stand scores increased significantly in the ES group (29 (8) to 38 (13)), compared to the Control group (29 (14) to 29 (13)) with interaction effects ($p < 0.01$). *Post hoc* analysis confirmed a significant increase in STS score in the ES group ($p < 0.01$) with no increase in the Control group ($p > 0.05$), and a large between group effect size for the difference in change scores of $g = 1.40$ (See Table 3.2 and Figure 3.14). There was no change in STS RPE in either group (see Table 3.2). There was a significant effect of time ($p < 0.01$) but no interaction effect for the 6MWT, with Control and ES groups increasing walk distance by 34 (19) and 44 (37) m respectively (See Table 3.2 and Figure 3.15). There was a significant increase in 6MWT RPE for the Control group (10 (2) to 12 (2)), Holm Bonferroni corrected $p < 0.05$) but not in the ES group, with an effect size of $g = 0.73$ for the difference in change scores between groups (see Table 3.2).

Table 3.2. Summary data of muscle function measures

	Group	Pre	Post	% Δ	F	p	g
STS score	Control	26 (14)	29 (13)	0%	9.75	.01	1.40
	ES	29 (8)	38 (13)**	31%			
STS RPE	Control	13 (3)	14 (2)	5%	0.14	.71	0.17
	ES	13 (2)	14 (2)	8%			
6MWT (m)	Control	435 (85)	468 (78)	8%	0.64	.43	0.36
	ES	449 (77)	493 (102)	10%			
6MWT RPE	Control	10 (3)	12 (2)	22%	2.63	.12	0.73
	ES	10 (3)	11 (3)	7%			

Abbreviations: ES; Exercise snacking group, STS; 60 second sit-to-stand test, RPE; Borg's rate of perceived exertion (6-20 scale), 6MWT; six minute walk test. Data presented as mean (SD), % Δ pre-to-post change within groups, F and p values for interaction effect from two-way repeated measures ANOVA, and Hedges g effect size of difference in change scores between groups. ** denotes significant interaction effect of $p < 0.01$.

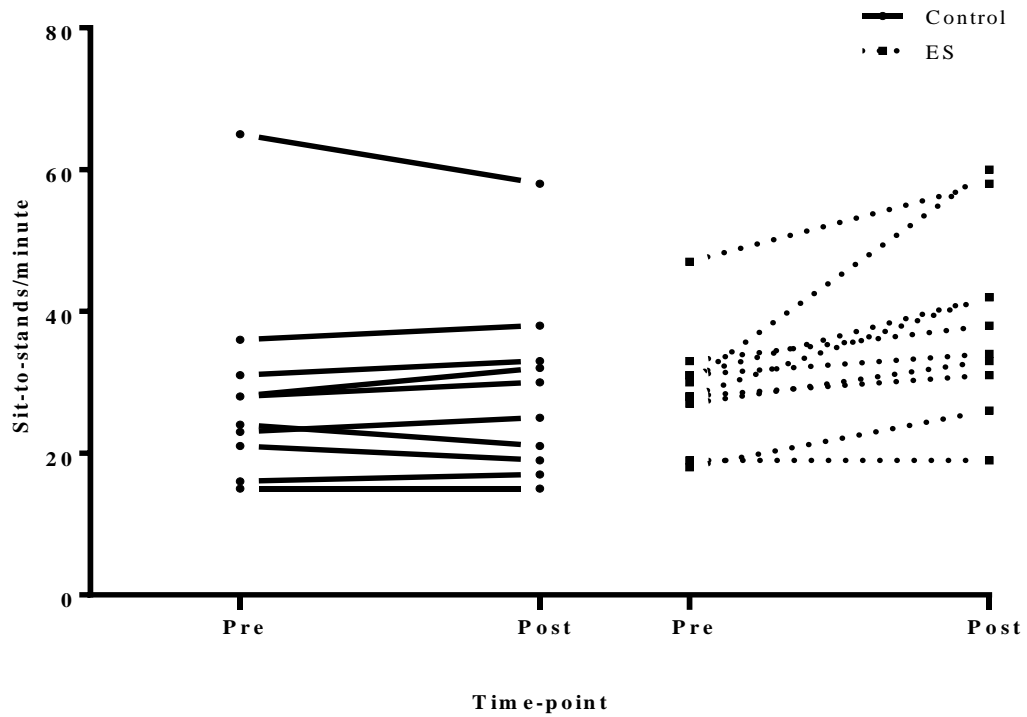


Figure 3.14. Individual changes in sit-to-stand score from pre- to post-intervention of either 28 days of yogurt at breakfast only (Control), or yogurt at breakfast and exercise snacking twice daily (ES).

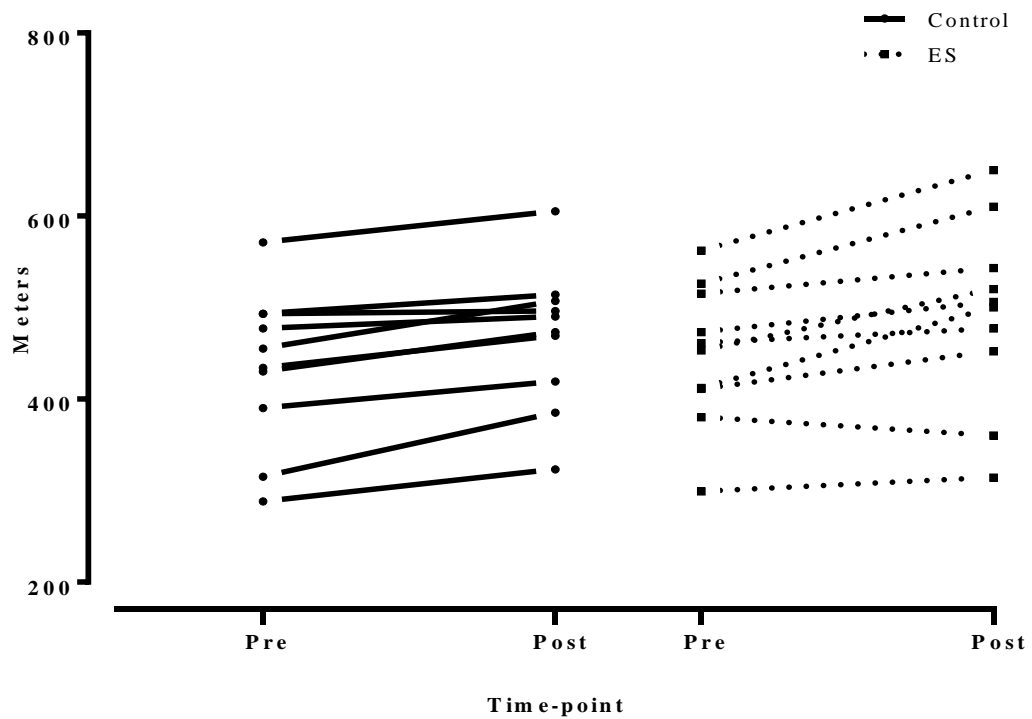


Figure 3.15. Individual changes in meters walked during a six minute walk test from pre- to post-intervention of either 28 days of yogurt at breakfast only (Control), or yogurt at breakfast and exercise snacking twice daily (ES).

3.3.2 Leg pressing and plantar flexion measures

There were no significant time or interaction effects for V_{\max} , F_{\max} , or P_{\max} (Table 3.3 and Figure 3.16). Effect sizes for difference in change scores between groups were moderate for V_{\max} and F_{\max} ($g = 0.62$ and $g = 0.49$ respectively) and large for P_{\max} ($g = 0.81$). A significant time effect ($p < 0.05$) was observed for $P_{\max 40}$, with no interaction effect (Table 3.3 and Figure 3.17). The effect size for the difference in the change scores between groups for $P_{\max 40}$ was large ($g = 0.94$).

There were no changes in MVF or ITR during isometric plantar flexion following the intervention period in either group (Table 3.3). One participant from the ES group was removed from the ITR data analysis due to inability to achieve >85% of MVF during the ITR trials.

Table 3.3. Summary data of power, strength, velocity and neural drive measures

	Group	Pre	Post	% Δ	F	<i>p</i>	<i>g</i>
V_{\max} (m/s)	Control	1.61 (0.29)	1.56 (0.24)	-3%	1.85	.19	0.62
	ES	1.75 (0.34)	1.81 (0.23)	3%			
F_{\max} (N)	Control	950 (290)	929 (170)	-2%	1.19	.29	0.49
	ES	984 (249)	1032 (289)	5%			
P_{\max} (W)	Control	370 (98)	363 (86)	-2%	3.26	.09	0.81
	ES	446 (170)	472 (166)	6%			
$P_{\max 40}$ (W)	Control	353 (81)	354 (69)	0%	4.38	.05	0.94
	ES	416 (161)	437 (158)	5%			
PF MVF (N)	Control	576 (91)	591 (118)	3%	0.29	.60	0.24
	ES	580 (172)	571 (207)	-2%			
PF ITR (%)	Control (9)	77 (28)	76 (25)	-2%	0.00	.96	0.03
	ES	81 (13)	79 (20)	-2%			

Abbreviations: ES; Exercise snacking group, V_{\max} ; extrapolated maximum leg pressing velocity, F_{\max} ; extrapolated maximum leg pressing force, P_{\max} ; interpolated maximum leg pressing power, $P_{\max 40}$; mean leg pressing power from eight discrete repetitions at 40% of one repetition maximum force, PF; plantar flexion MVF; maximum voluntary force, ITR; interpolated twitch ratio. Data presented as mean (SD), % Δ pre-to-post change within groups, F and *p* values for interaction effect from two-way repeated measures ANOVA, and Hedges *g* effect size of difference in change scores between groups. Group size $n = 10$ unless stated otherwise.

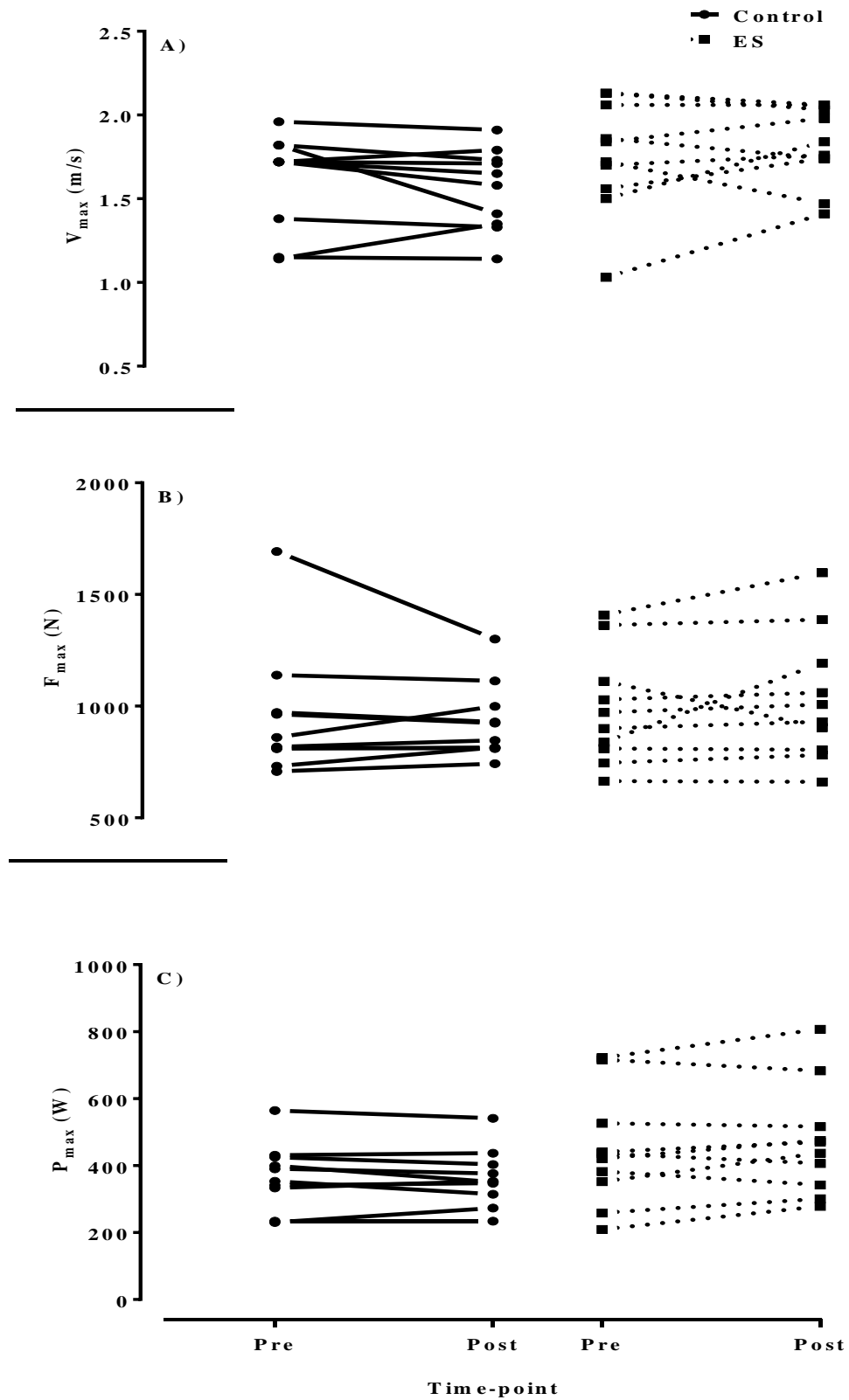


Figure 3.16. Individual changes in extrapolated peak A) velocity (V_{\max}), B) force (F_{\max}), and C) interpolated peak power (P_{\max}), pre- to post-intervention of either 28 days of yogurt at breakfast only (Control), or yogurt at breakfast and exercise snacking twice daily (ES).

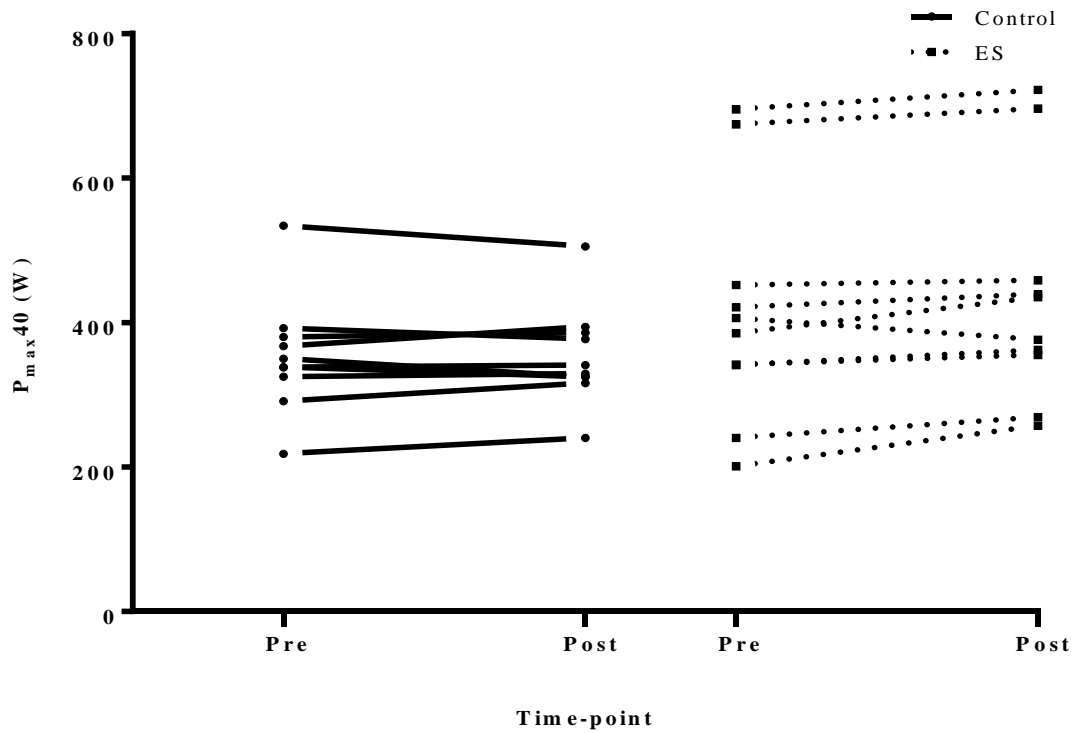


Figure 3.17. Individual changes in mean leg pressing power of 8 discrete repetitions against an external load of 40% of one repetition max from pre- to post-intervention of either 28 days of yogurt at breakfast only (Control), or yogurt at breakfast and exercise snacking twice daily (ES).

3.3.3 Postural sway during quiet standing

Table 3.4 displays summary data for all six trials with feet 15 cm apart, with eyes open and eyes closed trials pooled; there were no significant pre-to-post intervention changes between groups in postural sway (CoP mean speed, or AP range). When eyes open and closed conditions were pooled for the trials with feet together, AP range did not change significantly, and there was a significant interaction effect for CoP mean speed. *Post hoc* analysis revealed that there was a decrease in CoP mean speed in the Control group only (28.2 (10.3) to 23.5 (5.4) mm/s; $p < 0.05$; large between group effect size for difference in change scores of $g = 1.36$) (Table 3.4).

Summary data describing postural sway indices with eyes open and eyes closed during quiet standing are displayed in Table 3.5. There were no significant changes in measures of postural sway when feet were 15 cm apart, in either the eyes open or eyes closed conditions, and no significant differences between groups in either postural sway measure for trials with eyes open and feet together. In the feet together with eyes closed condition, there was a significant interaction effect for CoP mean speed, which *Post hoc* analysis revealed to be a decrease in CoP mean speed in the Control group only (34.4 (13.8) to 27.5 (8.6) mm/s; $p < 0.01$; large effect size for the difference in change scores between groups of $g = 1.75$).

When comparing the differences in postural sway between the eyes open and eyes closed conditions, from pre-to-post intervention, there was a significant interaction effect in for CoP mean speed in the feet together condition, with the ES group showing a significant increase (11.5 (11.3) to 15.6 (12.4) mm/s; $p < 0.05$; large between group effect size for the difference in change scores of $g = 1.46$). There were no other significant changes when comparing the difference in postural sway with eyes open versus closed, pre-to-post intervention.

Table 3.4. Summary data of postural sway during quiet standing data with eyes open and eyes closed conditions combined.

	Group	Pre	Post	% Δ	F	<i>p</i>	<i>g</i>
Feet apart							
CoP mean speed (mm/s)	Control	14.6 (2.9)	13.5 (1.6)	-8%	1.76	.20	0.63
	ES	15.0 (5.2)	15.0 (4.3)	0%			
AP range (mm)	Control	30.6 (12.1)	30.9 (10.1)	1%	1.88	.18	0.65
	ES	29.3 (4.1)	27.3 (5.1)	-7%			
Feet together							
CoP mean speed (mm/s)	Control	28.2 (10.3)	23.5 (5.4)	-16%	7.56	.02	1.36
	ES	25.0 (7.7)	27.1 (9.8)*	8%			
AP range (mm)	Control	44.0 (16.8)	40.7 (11.8)	-8%	1.22	.29	0.58
	ES	38.6 (9.1)	40.4 (7.7)	5%			

Abbreviations: ES; Exercise snacking group, Cop; centre of pressure, AP; anterior-posterior plane of motion. Data presented as mean (SD), % Δ pre-to-post change within groups, F and *p* values for interaction effect from two-way repeated measures ANOVA, and Hedges *g* effect size of difference in change scores between groups. * denotes significant interaction effect of *p* < 0.05.

Table 3.5. Summary data of postural sway during quiet standing data comparing eyes open and eyes closed conditions.

	Group	Pre	Post	% Δ	F	<i>p</i>	<i>g</i>
CoP mean speed (mm/s)							
Feet apart; eyes open	Control	13.1 (2.7)	12.0 (1.8)	-8%	1.83	.20	0.39
	ES	12.1 (3.1)	11.8 (2.8)	-3%			
Feet apart; eyes closed	Control	16.5 (3.4)	15.0 (2.2)	-9%	0.64	.44	0.57
	ES	17.9 (7.6)	18.2 (6.0)	2%			
Feet together; eyes open	Control	23.1 (7.6)	19.6 (3.7)	-15%	1.27	.29	0.92
	ES	19.3 (3.6)	18.8 (5.3)	-2%			
Feet together; eyes closed	Control	34.4 (13.8)	27.4 (8.6)	-20%	5.44	.03	1.75
	ES	30.0 (12.3)	33.6 (15.1)	12%*			
AP range (mm)							
Feet apart; eyes open	Control	29.5 (13.9)	28.1 (10.8)	-5%	0.02	.87	0.04
	ES	26.3 (3.4)	25.1 (4.3)	-5%			
Feet apart; eyes closed	Control	32.7 (12.1)	33.8 (10.7)	3%	2.29	.15	0.70
	ES	32.5 (6.2)	29.3 (6.6)	-10%			
Feet together; eyes open	Control	40.1 (16.6)	33.5 (13.8)	-17%	0.06	.81	0.48
	ES	35.5 (9.0)	34.4 (7.6)	-3%			
Feet together; eyes closed	Control	47.6 (19.9)	44.5 (10.0)	-7%	0.18	.68	0.73
	ES	41.4 (10.2)	45.4 (11.2)	10%			

Abbreviations: ES; Exercise snacking group, Cop; centre of pressure, AP; anterior-posterior plane of motion. Data presented as mean (SD), % Δ pre-to-post change within groups, F and *p* values for interaction effect from two-way repeated measures ANOVA, and Hedges *g* effect size of difference in change scores between groups. * denotes significant interaction effect of *p* < 0.05.

3.3.4 DXA

There were no significant changes in body mass, or DXA measured total percentage body fat, total fat mass, trunk fat mass, total lean mass, or lean leg mass, in either group, with effect sizes for the difference in the change scores between groups of $g = 0.48$, $g = 0.48$, $g = 0.09$, $g = 0.44$, and $g = 0.68$, respectively (Table 3.6 and Figure 3.18 (lean leg mass only)). One participant from the Control group was removed from DXA analysis due to movement artefact on one scan.

3.3.5 pQCT

Calf mCSA and muscle density measured by pQCT did not change significantly for either group, with effect sizes of $g = 0.10$ and $g = 0.49$ for the between group difference in change scores respectively. There were significant time and interaction effects for pQCT measured calf fat CSA. In the ES group there were significant reductions in calf fat CSA (2765 (1123) to 2712 (1106) mm²; Holm Bonferroni corrected $p < 0.01$) with no change in the Control group (2380 (636) to 2373 (644) mm²), with a large effect size for the difference in change scores between groups ($g = 1.00$) (Table 3.7 and Figure 3.19). One participant from the ES group was removed from the calf pQCT scan analysis due to movement artefact.

There were no significant changes in pQCT thigh scan variables in either group. Effect sizes for the between group difference in changes scores were large for thigh mCSA ($g = 0.96$), moderate for thigh fat CSA ($g = 0.75$), and very small for thigh muscle density ($g = 0.05$) (Table 3.7 and Figure 3.19). One participant from the Control group was removed from the thigh pQCT scan analysis due to movement artefact.

Table 3.6. Summary data of body mass and dual energy x-ray absorptiometry measures

	Group	Pre	Post	% Δ	F	<i>p</i>	<i>g</i>
Body mass (kg)	Control	70.5 (11.2)	70.3 (11.4)	0%	0.23	.64	0.27
	ES	69.0 (10.0)	69.0 (10.0)	0%			
% body fat	Control (<i>n</i> =9)	35.1 (7.4)	35.2 (6.8)	0%	0.95	.34	0.48
	ES	34.0 (7.0)	33.7 (7.0)	-1%			
Fat mass (kg)	Control (<i>n</i> =9)	24.4 (6.7)	24.5 (6.6)	0%	1.03	.33	0.48
	ES	23.4 (7.1)	23.2 (7.1)	-1%			
Trunk fat mass (kg)	Control (<i>n</i> =9)	12.3 (4.8)	12.4 (4.8)	0%	0.04	.86	0.09
	ES	11.9 (4.9)	11.9 (4.8)	0%			
Lean mass (kg)	Control (<i>n</i> =9)	44.8 (8.6)	44.6 (8.4)	0%	0.86	.37	0.44
	ES	44.9 (6.5)	45.0 (6.4)	0%			
Leg lean mass (kg)	Control (<i>n</i> =9)	15.3 (2.4)	15.3 (2.3)	0%	2.06	.17	0.68
	ES	15.3 (2.0)	15.5 (2.2)	1%			

Abbreviations: ES; Exercise snacking group. Data presented as mean (SD), %Δ pre-to-post change within groups, F and *p* values for interaction effect from two-way repeated measures ANOVA, and Hedges *g* effect size of difference in change scores between groups. Group size *n* = 10 unless stated otherwise.

Table 3.7. Summary data of peripheral quantitative computed tomography and ultrasound measures.

	Group	Pre	Post	% Δ	F	<i>p</i>	<i>g</i>
pQCT calf mCSA (mm²)	Control	6610 (972)	6616 (964)	0%	0.04	.84	0.10
	ES (<i>n</i> =9)	6569 (928)	6561 (905)	0%			
pQCT calf fCSA (mm²)	Control	2380 (636)	2373 (644)	0%	4.55	.04	1.00
	ES (<i>n</i> =9)	2765 (1123)	2712 (1106)*	-2%			
pQCT calf density (mg/mm³)	Control	74.3 (1.9)	74.4 (2.1)	0%	1.25	.28	0.49
	ES (<i>n</i> =9)	75.1 (2.1)	75.5 (1.9)	1%			
pQCT thigh mCSA (mm²)	Control (<i>n</i> =9)	5682 (912)	5680 (959)	0%	3.90	.07	0.94
	ES	5390 (819)	5520 (920)	2%			
pQCT thigh fCSA (mm²)	Control (<i>n</i> =9)	6305 (1810)	6305 (1828)	0%	2.49	.13	0.75
	ES	6103 (1820)	5996 (1875)	-2%			
pQCT thigh density (mg/cm³)	Control (<i>n</i> =9)	68.2 (2.2)	68.9 (1.2)	1%	0.01	.93	0.05
	ES	68.9 (2.2)	69.4 (2.5)	1%			

Abbreviations: ES; Exercise snacking group, pQCT; peripheral quantitative computed tomography (calf at scanned at 66% tibia length medially, thigh scanned at 25% femur length medially), mCSA; muscle cross sectional area, fCSA; fat cross sectional area. Data presented as mean (SD), %Δ pre-to-post change within groups, F and *p* values for interaction effect from two-way repeated measures ANOVA, and Hedges *g* effect size of difference in change scores between groups. Group size *n* = 10 unless stated otherwise. * denotes significant interaction effect of *p* < 0.05.

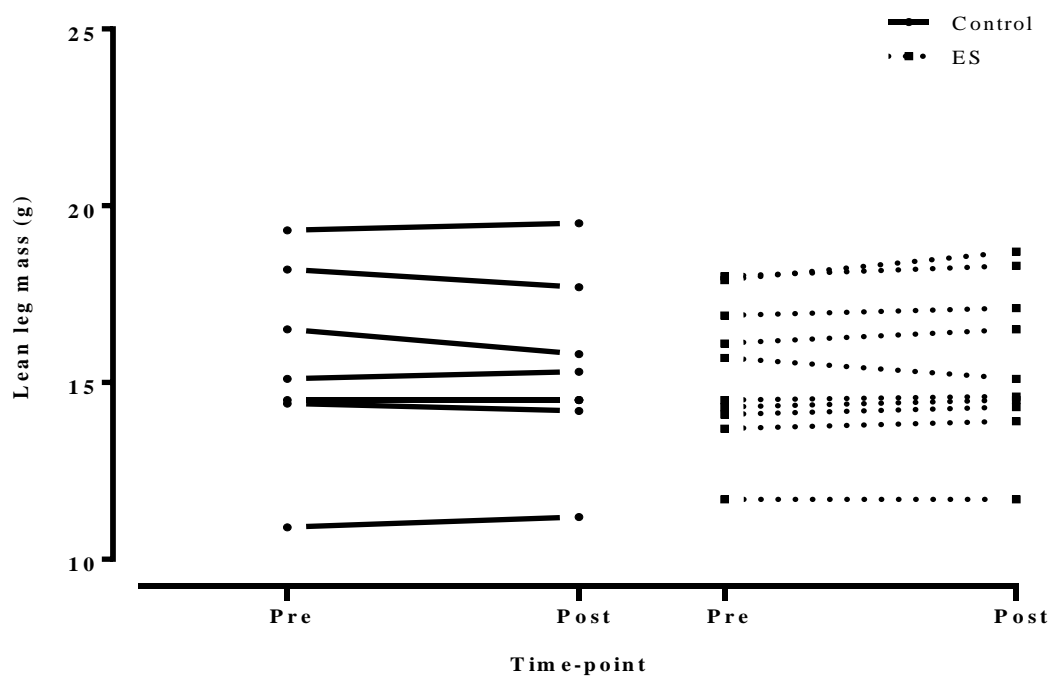


Figure 3.18. Individual changes in DXA measured lean leg mass from pre- to post-intervention of either 28 days of yogurt at breakfast only (Control), or yogurt at breakfast and exercise snacking twice daily (ES).

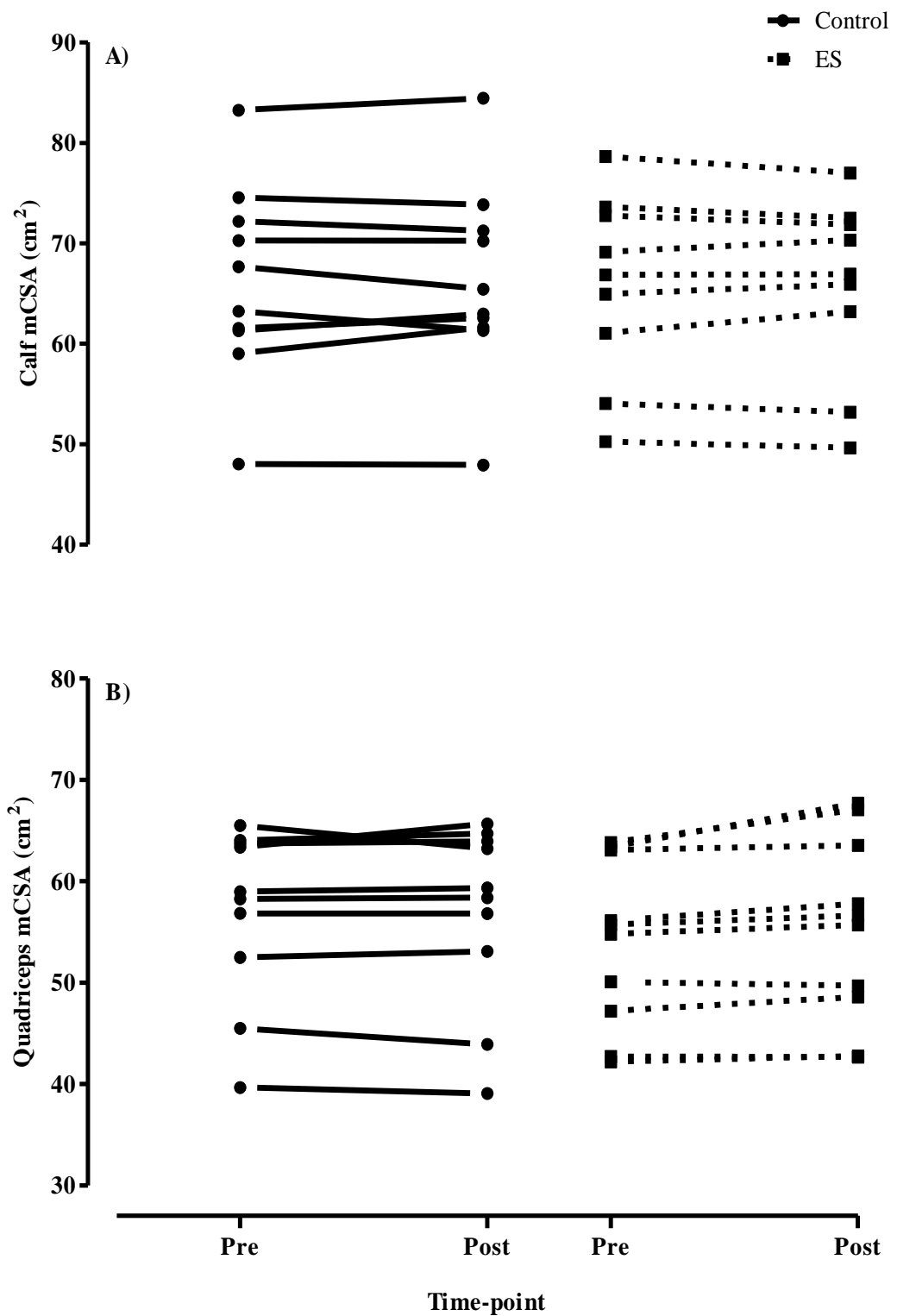


Figure 3.19. Individual changes in pQCT measured A) calf muscle at 66% tibia, and B) thigh muscle group at 25% femur, pre- to post-intervention of either 28 days of yogurt at breakfast only (Control), or yogurt at breakfast and exercise snacking twice daily (ES).

3.3.6 Ultrasound

There were no significant changes in muscle fascicle length or pennation angle of either the MG or VL, and in either group. All the effect sizes were small for the between group difference in change scores (Table 3.8). Fascicle length in the MG of the Control group was 28 (4) mm pre-intervention, and 24 (5) mm post-intervention, and in the VL were 26 (6) and 28 (9) mm pre- and post-intervention respectively. For the ES group, MG fascicle length was 29 (4) and 28 (4) mm pre- and post-intervention respectively, and for VL was 35 (14) and 33 (14) mm pre- and post-intervention respectively. Typical absolute error of the measurement (95% confidence intervals) for fascicle length when the same images were assessed three times were 1.61 (1.30-2.19) and 1.76 (1.40-2.38) mm for the MG and VL respectively. For the Control group, pennation angle of the MG was 19 (2) ° and 20 (4) °, and of the VL was 17 (2) ° and 17 (3) °, pre- and post-intervention respectively. The pennation angle of the MG in ES group was 19 (3) ° pre-, and 19 (4) ° post-intervention, and in the VL was 16 (4) and 18 (4) ° pre- and post-intervention respectively. Typical absolute error of the measurement for pennation angle was 0.93 (0.75-1.26) ° and 0.98 (0.78-1.32) ° for the MG and VL respectively. One participant from the ES group was removed from the ultrasound thigh scan analysis due to no discernible muscle fascicles being in view on the images recorded at either pre- or post-intervention time points.

Table 3.8. Summary data of ultrasound measures.

	Group	Pre	Post	% Δ	F	<i>p</i>	<i>g</i>
US GM fascicle length (mm)	Control	28 (4)	24 (5)	-14%	0.94	.35	0.45
	ES	29 (4)	28 (4)	-4%			
US GM fascicle pennation angle (°)	Control	19 (2)	20 (4)	4%	0.36	.56	0.28
	ES	19 (3)	19 (2)	-1%			
US VL fascicle length (mm)	Control	26 (6)	28 (9)	11%	2.19	.16	0.69
	ES (<i>n</i> =9)	35 (14)	33 (13)	-7.5%			
US VL fascicle pennation angle (°)	Control	17 (2)	17 (3)	-4%	1.64	.22	0.59
	ES (<i>n</i> =9)	16 (4)	18 (4)	9%			

Abbreviations: ES; Exercise snacking group, US; ultrasound, GM; *gastrocnemius medialis* muscle belly, VL; *vastus lateralis* at mid-thigh. Data presented as mean (SD), %Δ pre-to-post change within groups, F and *p* values for interaction effect from two-way repeated measures ANOVA, and Hedges *g* effect size of difference in change scores between groups. Group size *n* = 10 unless stated otherwise.

3.3.7 Physical Activity and diet

There were no changes in PAL in either group from baseline assessment to the last week of the intervention, nor were there changes in total calorie (kcal/kg/day) or carbohydrate intake (g/kg/day). There were significant time effects for protein ($p < 0.01$) and fat intake ($p < 0.05$), with no differences between groups for protein or fat intake (see Table 3.9).

Table 3.9. Summary data of dietary intake from three-day diet records pre-intervention and during the last week of intervention

	Group	Pre	During	% Δ	F	<i>p</i>	<i>g</i>
Energy intake (kcal/kg/day)	Control	28 (7)	26 (6)	-9%	0.12	.73	0.18
	ES	28 (6)	27 (9)	-4%			
CHO intake (g/kg/day)	Control	3.02 (0.88)	2.82 (0.84)	-7%	0.04	.84	0.09
	ES	2.95 (1.21)	2.66 (0.85)	-10%			
PRO intake (g/kg/day)	Control	1.01 (0.19)	1.17 (0.30)	17%	0.13	.73	0.16
	ES	1.10 (0.21)	1.30 (0.34)	19%			
Fat intake (g/kg/day)	Control	1.11 (0.47)	0.83 (0.28)	-25%	0.18	.67	0.19
	ES	1.32 (0.40)	1.11 (0.40)	-15%			

Abbreviations: ES; Exercise snacking group, CHO; carbohydrates, PRO; protein. Data presented as mean (SD), %Δ pre-to-post change within groups, F and *p* values for interaction effect from two-way repeated measures ANOVA, and Hedges *g* effect size of difference in change scores between groups.

3.4 Discussion

The impact of undertaking 28 days of twice daily homebased exercise snacking, supplemented with 150 g of yogurt at breakfast, on lower limb muscle function and anthropometry was explored in healthy older adults. Adherence to the exercise regime was very high (98%), and participants in the ES group showed marked improvements in the number of sit-to-stands performed in 60 seconds, with no improvement in the Control group. Large effect sizes were also observed for the between group differences in the change scores for leg press P_{\max} and $P_{\max40}$, and thigh mCSA.

The exercise snacking regime consisted of five leg exercises, each completed twice a day spread over two bouts, with the aim to complete as many repetitions of each exercise as possible in a minute with no external load above bodyweight. This mode of exercise deviates from previously explored successful homebased exercise programmes, primarily in that all exercise was performed with no extra load above bodyweight, participants undertook exercise twice a day, there were no supervised exercise sessions in the home, and the programme lasted only four weeks (Thiebaud *et al.* 2014). The ES group significantly improved STS score, with the 31% improvement in the STS in 60 seconds in the present pilot study being remarkably similar to the 30% improvement in 30 second STS score observed after six weeks resistance training in older adults by Cavani *et al.* (2002). Perhaps more surprisingly given the mode of training, moderate improvements in leg press V_{\max} and F_{\max} of 3% and 5% respectively were also observed. There were also trends towards significant increases in P_{\max} (6% increase, $p = 0.088$) and $P_{\max40}$ (5% increase, $p = 0.051$). As a point of comparison, in the study by Bean *et al.* (2004), older adults trained three times a week for 12 weeks, completing three sets of 10 maximum concentric contraction speed repetitions of six similar exercises to those of the present pilot study whilst wearing a weighted vest. The weighted vest group increased maximum leg press power by 12% assessed with Keiser leg press dynamometry, whereas in a third of the programme duration and without external loading, the present pilot study saw increases in P_{\max} of 6%. The weighted vest group also increased power at 40% of 1-RM by 23%, whereas in the present pilot study $P_{\max40}$ increased by 5%. It should be noted that the large increase in power at 40% of 1-RM observed by Bean *et al.* (2004) was not statistically significant, likely due to a large degree of variability in the

measurement. In the present pilot study, $P_{\max}40$ was taken as the mean of 8 repetitions, so is perhaps a more reliable measure of leg muscle power. Another possible cause of the disparity in degree of low load power increases between studies may be due to lack of progressive increase in external loading in the present pilot study. Whilst training volume of each exercise snack might increase with more reps performed per exercise bout over the course of the intervention, in the study by Bean *et al.* (2004) 2% bodyweight was added to the mass of the vest after every successfully completed training session. This increased loading may have had a greater impact on ability to generate power with training than the increased speed of repetitions alone that would be needed to perform more repetitions per bout exercise snacking (Raj *et al.* 2010). Whether functional improvements of the exercise snacking regime over longer training durations would continue to increase with the only element of progression being completion of more repetitions in a minute could be the focus of future work. Nonetheless, the findings represent changes with real world relevance given the estimated annual loss of muscle strength of 1-5% described by Seene and Kaasik (2012), particularly as the strength and power gains were achieved in four weeks with a zero cost exercise intervention.

Whilst the exercise snacking was highly effective in improving STS score, and moderately effective for improving lower limb strength and power, other measures of muscle function were not changed. Specifically, 6MWT distance, and isometric plantar flexion MVF and ITR, were not improved by exercise snacking. This is maybe not surprising given these two measures were arguably less specific to the exercises performed as part of the exercise snacking programme (Baker *et al.* 1994). Both groups improved 6MWT from pre-to-post intervention. In favour of the exercise snacking regimes efficacy, there was a moderate-to-high effect size for the difference in 6MWT RPE change score ($g = 0.73$) suggesting that despite both groups walking further in six minutes in post-intervention testing, the ES group did so with less perceived exertion than the Control group. It may have been that lack of familiarisation to the 6MWT resulted in both groups increasing distance covered. Compared to previous literature, distances covered in the 6MWT of the previous study are low (Ng *et al.* 2011). This was likely due to the truncated walkway distance of 10 m, and does not reflect the functional capacity of the study population. Furthermore, participants were instructed to walk at a self-selected speed that was comfortable, but to aim to

cover the maximum distance possible in 6 minutes, however with RPE scores of 10-12 both pre- and post-intervention, participants did not perceive the test to be strenuous (with 11 being defined at 'light' (Borg and Dahlstrom 1962)). The subjectivity associated with the instructions, the lack of familiarity with the test, and the relative ease with which the test was completed suggest that the 6MWT may have been inappropriate to distinguish responses to the exercise snacking intervention in the population used in the present pilot study. Moreover, the physical activity data suggest that the exercise snacking intervention did not increase physical activity levels, assessed as PAL, either directly or indirectly, so participants were unlikely to be adding any walking specific activity to their daily routine that might confer an increased 6MWT score. The physical activity data was recorded in one-minute-epochs, and in subsequent inspection of individual daily physical activity counts bouts of exercise snacking were not identifiable. Any increase in muscle strength or power did not lead to participants being any more active in their day to day life, in the week of physical activity recording. Of course, the risk that the monitoring itself caused participants to alter their physical activity behaviours in either the pre-intervention or during the last week of the intervention should not be ruled out.

There was no improvement in isometric plantar flexion MVF or ITR in either group. Maximum force generating capacity was not trained during the exercise snacking regime, and there appears to have been no increase in calf muscle size, so it is unsurprising that there was no change in MVF. However, given that there was an increase in F_{\max} and P_{\max} , neural adaptations such as increased neural drive may have been anticipated (Scaglioni *et al.* 2002), particularly given that adaption to strength training is led by neurological changes (Folland and Williams 2007a). Whilst two familiarisations were undertaken, older adults who do not undertake regular resistance training are typically unaccustomed to producing maximal effort of isolated muscle groups (Shield and Zhou 2004). Furthermore, a single stimulation pulse was used due to the associated discomfort of the stimulation in an older population, which may not have been as reliable as doublets or trains of twitch pulses (Folland and Williams 2007b). Equally, as percutaneous stimulation was delivered to the nerve trunk with surface electrodes, there is the possibility that antagonist muscle groups could have been stimulated at the instant of twitching (Shield and Zhou 2004). Should antagonists be sufficiently activated such that the agonist twitch was masked, this would falsely

give the impression that the muscle was fully activated. Given that activation was $\approx 75\text{--}80\%$ this was unlikely to be the case, however, comparable plantar flexion activation accompanied by $\approx 10\%$ co-activation was observed in healthy older males by Morse *et al.* (2005). Moreover, it may be that given limited stimulus for neurological adaptation in the exercise snacking intervention, and the methodological challenges associated with the measurement of isometric plantar flexion MVF and ITR, particularly in older adults, as demonstrated by the variability in the data from the control group, detecting neural adaptations was perhaps unlikely in the present pilot study.

In concordance with the lack of direct evidence for an improvement in neural drive, and despite the intuitive appeal of improved balance with increased muscle strength, there was no effect on postural sway (CoP mean speed, or AP range) during quiet standing in the present pilot study. In some cases, there was in fact a worsening of postural sway in the ES group compared to the Control group, specifically relating to CoP speed during quiet standing with feet together. With feet together during 60 s of quiet standing, CoP mean speed, when combining both trials (eyes open and closed conditions), and in the eyes closed trial, decreased significantly in the Control group (16% and 20% respectively), compared to non-significant increases in these trials of 8% and 12% in the ES group. Furthermore, from the feet together with eyes closed trial, it would also seem that there was actually a worsening of vestibular balance, insofar as the difference in CoP mean speed between eyes open and eyes closed conditions with the feet together increased by 35% in the ES group, compared to reducing by 28% in the Control group. However, this should be kept in context; this trend was only evident in one of the conditions. A recent meta-analysis by Muehlbauer *et al.* (2015) evidenced that the relationship between balance and leg strength/power is in fact only weak and such should be tested and trained separately. Lin *et al.* (2008) demonstrated CoP mean speed to be a reliable measure in older adults, so perhaps the improved balance during quiet standing in the control group could be attributed to a learning effect for the test. However, the apparent increase in postural sway in the ES group is counterintuitive, with no clear explanation. The exercise snacking training programme did not include any specific balance training exercises, and for unilateral exercises, participants could hold a chair or door frame for support, thus not overtly challenging balance. The most recent Cochrane review on exercise for improving balance in older adults suggests that evidence for strength training alone improving

balance is also weak (Howe *et al.* 2011). With the limited sample size, short duration of training with a deliberately low training stimulus, and as such only moderate improvements in strength and power observed in the present pilot study, the lack of improvement in balance could also have been expected. Further studies to improve the efficacy of the exercise snacking regime could look to include a deliberate balance training component, separate to the strength training aspect of the regime.

There were some small but noteworthy changes in anthropometric measures of the legs following the ES intervention. In particular, leg lean mass measured by DXA increased by 1% ($g = 0.68$), and thigh mCSA increased by 2% with a large effect size ($g = 0.98$). In comparison to effect size of 0.39 (0.17) (95% CI:0.05, 0.73) calculated in a meta-analysis by Schoenfeld *et al.* (2016b) for hypertrophy induced by low load resistance exercise training in 251 untrained males and female (of which 56 were 50+ years, the rest were 18-29 years), the present pilot study found greater gains in muscle size than was expected. The precise cause for this is not clear, but could be in part due to the very high frequency of training, although this can only be speculated due to the scarcity of studies using twice daily exercise programmes (Schoenfeld *et al.* 2016a; Dankel *et al.* 2016). It is also possible that the hypertrophy observed in the present pilot study was in part due to the additional protein ingested at breakfast via the yogurt supplement. This notion would be supported by the evidence of Mamerow *et al.* (2014) that 24-hour MPS is greater with an even distribution of protein throughout the day compared to a more traditional, evening heavy, protein distribution. Whilst it is encouraging that two independent measurements techniques gave concomitant findings for leg muscle size, another potential cause of the observed increase in muscle size post-intervention may have been exercise induced oedema from the previous day's exercises (Damas *et al.* 2015). However, this seems unlikely given that the exercise snacking bouts provided only a moderate training stimulus, the participants would have been accustomed to the exercise after 28 days, and the increase in mCSA was not observed in the calf muscle group that was also measured by pQCT (DeFreitas *et al.* 2011). Overall, a reasonable degree of confidence can be placed in the exercise snacking intervention having a beneficial impact on muscle cross sectional area of the thigh muscle.

The ultrasound measures of fascicle characteristics are not entirely logical, with seemingly non-physiological values for VL fascicle length observed compared

to previous literature (Kwah *et al.* 2013). Despite VL fascicle pennation angles being congruent with data reported previously, VL fascicle lengths in the present pilot study of 26-35 mm appear to be markedly too low, with normal values ranging from 50-100 mm at the mid-point of the muscle (Kwah *et al.* 2013). Even in comparison to older adults undertaking six weeks of progressive resistance training, VL fascicle length was only a third of the length of that reported by Scanlon *et al.* (2014). Moreover, whilst the relative changes seem physiologically plausible compare to the findings of Scanlon *et al.* (2014), fascicle length would be expected to increase with increased mCSA, not decrease as was observed in the present pilot study (Folland and Williams 2007a). Confusingly, pennation angle changes in the VL would seem to align with the apparent hypertrophy of the quadriceps, although it is of course not confirmed that VL specifically underwent hypertrophy given the inability to isolate single muscles on a pQCT image (Erlandson *et al.* 2016). A further limitation of attempting to align the US and pQCT data is that the pQCT scan site was 25% of femur length, but the US scan site was 50% thigh length to increase image clarity of the VL specifically. Moreover, the degree of variability in data reported in both the control and ES groups of the present pilot study suggest that little confidence can be given to the present findings of regarding muscle fascicle characteristics.

Both groups increased daily protein intake per kg body mass by 17 % and 19% (Control and ES respectively) during the intervention, going from 1.05 (0.20) g/kg/day to 1.24 (0.32) g/kg/day (pooled mean). Whilst the participants in the present pilot study were previously consuming over the recommended daily allowance (RDA) of protein, Phillips *et al.* (2016) present a convincing rationale that the RDA may not represent an optimal daily protein intake for older adults. The suggested range of 1.2 to 1.6 g/kg/day as a more appropriate daily protein intake was achieved in the present pilot study with the addition of 16.5 g of dairy protein at breakfast. On further inspection of the absolute increase in protein intake per day, intake increased by ≈ 10 g/day, suggesting that the extra protein at breakfast was more than was required to increase total protein intake to 'optimal' levels. That there was not an increase in daily protein intake equal to the amount contained in the yogurt is presumably because protein that would have been included in the breakfast meal, or at other meals later in the day, may have been replaced with the yogurt. It should be noted that this increase in daily protein was not accompanied with an increase in daily caloric intake, and although non-

significant, when pooling both groups data, mean daily fat intake showed a trend for a decrease by $\approx 20\%$ with a moderate effect size ($g = 0.60$). In any case, despite achieving over 1.2 g/kg/day of protein there was no increase in strength or hypertrophy in the Control group. This supports the work of Kim *et al.* (2017), suggesting in that adjusting protein distribution without the addition of exercise does not increase muscle mass or strength, and further highlighting the importance of a combination of exercise and nutrition to address muscle loss with ageing. However, given the short duration of the intervention, and the use of only three day diet records that are often criticised for a bias towards under reporting (Macdiarmid and Blundell 1998), these data should be kept in context. Longer term increases in dietary protein intake in older adults may still hold potential health and body composition benefits, particularly if associated with reduced caloric intake (Halton and Hu 2004).

There are number of crucial considerations when contextualising the findings of the present pilot study, not least that the study design cannot support the efficacy of exercise snacking without dietary protein supplementation. The addition of two further groups (a true non-exercising control group, and an exercise snacking without yogurt group) would shed light on the importance of the additional protein at breakfast, but would require a large increase in sample size. Future studies of the exercise snacking regime should give further attention to the real-world applicability. Whilst the adherence to the exercise programme in this pilot study was very high, it cannot be assumed that this would persist longer than four weeks, or in other older populations (Picorelli *et al.* 2014). It should also be noted that the whilst the participants in the present pilot study were healthy and not functionally impaired, they were previously undertaking no regular structured exercise, so were perhaps more physiologically receptive to the training stimulus provided than a frail or clinical population might be (Gill *et al.* 2002). Investigation of physiological mechanisms to support the strength and hypertrophic gains observed in the present pilot study may allow for further optimisation of the exercise regime itself, creating a potentially more efficacious training stimulus. It should of course not be overlooked that high load resistance training has superior effects on increasing muscle mass and strength (Van Roie *et al.* 2013; Schoenfeld *et al.* 2016b). However, given that access and cost of exercise participation, and lack of knowledge of exercise modalities, are often key barriers to

exercise in older adults (Rasinaho *et al.* 2007), the very simple and homebased exercise snacking regime is a promising strategy.

In conclusion, the present pilot study highlights the potential efficacy of a 28-day, homebased exercise programme, supplemented with additional protein at breakfast for improving leg strength and muscle size in healthy older adults. Along with marked task specific improvements in sit-to-stand score, increases in leg press force and power production of 5% and 6% respectively demonstrate transferability of the training, and represent potentially clinically relevant improvements in function. More research is needed to understand the mechanisms by which the exercise snacking regime improves muscle strength and size, the role that supplemental protein plays in the hypertrophy observed, and the real-world acceptability.

Chapter 4: Exercise snacking to attenuate loss of muscle function during two weeks of step-reduction in healthy older men- A feasibility study

4.1 Introduction

Physical activity has been shown to be vitally important for maintaining muscle mass and function into older age (Booth and Zwetsloot 2010). During periods of dramatically reduced activity, such as bed rest, muscles of healthy individuals rapidly lose size, strength and power, most notably from the legs (Ferrando *et al.* 1996; Trappe *et al.* 2008). Evidence from Breen *et al.* (2013) has suggested that in healthy older adults, even reduced activity through step-reduction (to <1,500 steps/day for two weeks) leads to pronounced loss of lean mass from the legs of around 4%. However, in overweight older adults undertaking step-reduction to <1,000 steps/day for two weeks, no significant loss of lean mass was observed (McGlory *et al.* 2017).

During ageing, strength is typically lost more rapidly than would be expected based on the rate of muscle size loss (Clark and Manini 2008), and strength loss is synonymous with total unloading of the lower limbs (Kortebein *et al.* 2008; Rejc *et al.* 2015). However, an interesting finding from Breen *et al.* (2013) and McGlory *et al.* (2017) was that isometric knee extensor muscle strength was not reduced with step-reduction in older adults. It should be noted that in both of these previous studies, muscle strength was measured by unilateral isometric knee extensor torque using a dynamometer, for which extensive familiarisation has been demonstrated to be crucial to obtain reliable results (Ordway *et al.* 2006). Moreover, other components of physical function that have been shown to be affected by muscle disuse were not measured, such as leg power (Rejc *et al.* 2015), neural drive (Kawakami *et al.* 2001), and balance (Kouzaki *et al.* 2007).

Devries *et al.* (2015) demonstrated that thrice weekly laboratory-based low-load resistance training in fact increased leg lean mass and strength in older men during two weeks of step-reduction to <1,500 steps/day. However, restricted access to resistance exercise equipment may render this exercise strategy infeasible, thus home based, body weight exercise may be a practical alternative to low load resistance training. As demonstrated in Chapter 3, the Exercise Snacking regime has potential to increase leg muscle power and size, and with high adherence rates, may be a suitable

exercise strategy to employ during periods of step-reduction. Reducing the extent of muscle function loss during periods of unloading would have clear implications for improving recovery after reduced activity periods. Moreover, with only the study of McGlory *et al.* (2017) reporting indices of recovery from step-reduction following a return to baseline habitual physical activity for two weeks post step-reduction, the efficacy of a more focussed programme of resistance training based recovery is unknown. Indeed, whether efforts to maintain muscle mass and function during periods of reduced activity might further improve the benefits of a subsequent retraining programme is also unclear.

The primary aim of the present feasibility study was to determine whether exercise snacking may have a protective effect against loss of lower limb skeletal muscle power in healthy males aged 65-80 years undertaking two weeks of step-reduction. Alongside this, the influence of exercise snacking during step-reduction on other indices of muscle function (maximum leg press velocity and strength, and neural drive during isometric plantar flexion), standing balance, anthropometric measures, and dietary intake were assessed. Thereafter, the possible benefits of the exercise interventions on recovery of muscle function and size was investigated over a short period of progressive resistance exercise training. It was hypothesised that step-reduction without exercise snacking would lead to marked loss of leg pressing power, along with lower limb muscle mass and cross-sectional area, but that thrice daily exercise snacking would offset this loss in muscle function and size. It was anticipated that whilst reductions in neural drive would also be observed due to step-reduction, standing balance would not be effected.

4.2 Methods

4.2.1 Participants

Eight healthy, community dwelling, older men (65-80 years) were recruited for the present study through posters displayed locally, and newspaper, radio, and social media, advertisement. Potential participants were fully informed as to the study design and methodology with written information, and an informal telephone interview was conducted to ascertain initial eligibility. The potential feasibility of that individuals participation in the study was discussed, specifically with respect to the logistical challenge of limiting daily steps to <1,500 for two weeks. Individuals who were non-smokers, BMI $\geq 20 < 30$ kg/m², had no contraindications to exercise, recent history of musculoskeletal injury, bleeding disorders, or adverse reactions to lidocaine anaesthetic, and were not regularly consuming any analgesic, anticoagulant, anti-inflammatory, or protein metabolism altering medications were invited to a screening assessment. Individuals were required to score 8 or above on the SPPB (Guralnik *et al.* 1994), with no score of zero on any components of the test at the initial screening. Thereafter, individuals habitually walking >3,500 steps/day, assessed for seven days by pedometer wear following the screening, were invited to take part in the study. All participants provided written informed consent. The NHS South West Cornwall and Plymouth REC approved the study, with an allocated REC reference number: 15/SW/0130. The project was subsequently registered as a clinical trial (ClinicalTrials.gov: NCT02495727). Participant characteristics are presented in Table 4.1.

Table 4.1. Participant characteristics

	SR (<i>n</i> = 3)	SR+ES (<i>n</i> = 5)
Age, years	71 (3)	71 (3)
Weight, kg	76.4 (10.8)	77.6 (4.2)
BMI, kg/m²	27.2 (3.0)	26.8 (2.0)
SPPB score	12 (0)	12 (0)
Daily steps/day at baseline	7455 (2061)	8694 (4806)

Data presented as mean (standard deviation). SR: Step-Reduction only group, SR-ES: Exercise-snacking with step-reduction group, BMI: Body mass index, SPPB: Short physical performance battery.

4.2.2 Procedures

Participants visited the laboratory on fourteen occasions throughout the trial. Following the initial screening visit, participants completed a three-day food diary and seven days of physical activity monitor wear (pedometer; CW600 Digi-Walker, Yamax, Tokyo, Japan, and arm-mounted physical activity monitor; SenseWear, BodyMedia, Inc., Pittsburg, PA, USA). At least 8 days after the screening visit, participants undertook a familiarisation with functional measures (Keiser leg press and plantar flexion twitch interpolation), and second familiarisation five to 14 days later. Between five to 14 days after the second familiarisation, the pre-intervention main trial took place, followed by 14 days of step-reduction, and the post-intervention main trial the day after. Three days after the post-intervention main trial, participants attended the first of six retraining sessions within 14 days after the first session. Thereafter, a final assessment of anthropometric measures and muscle function outcomes, using methods described previously, was undertaken three days after the final retraining session. A schematic overview of the study timeline is provided in Figure 4.1.

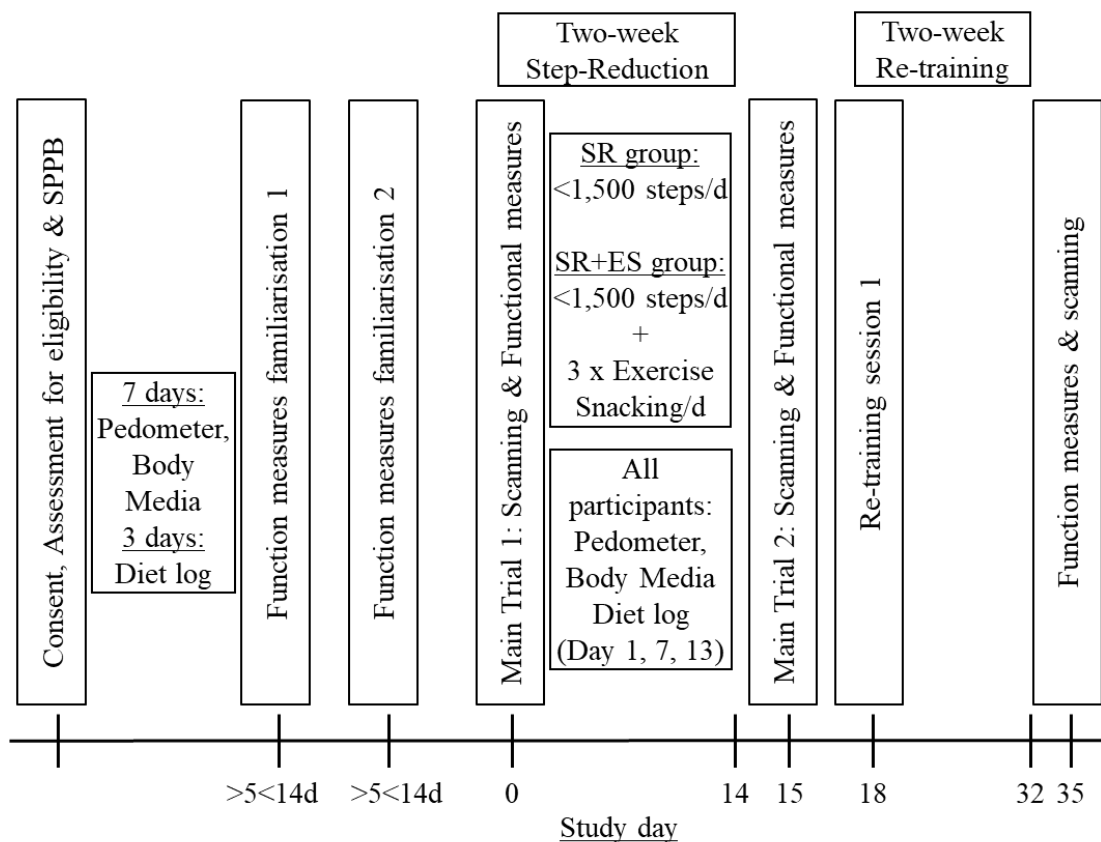


Figure 4.1. Schematic overview of the study timeline (d = day; SPPB = Short physical performance battery)

For pre- and post-step-reduction trial days, participants arrived at the laboratory following a 10 hour overnight fast wearing light clothing with no metal components, having drunk 1 pint of water between waking and arrival. After participants had voided, as per methods described in Chapter 3, weight and height were recorded, and DXA, and calf and thigh pQCT scans were performed, with an additional pQCT scan taken at 50% of femur length. Participants were provided with a mixed-macronutrient liquid meal of 2 g/kg body mass (BM) of carbohydrate; 0.8 g/kg BM of fat; 0.4 g/kg BM of protein, and *ad libitum* water. Following three hours of supine or seated rest, participants undertook muscle function measures. First, participants undertook the same standing balance test protocol as described in Chapter 3, with CoP mean speed and AP range assessed whilst standing on a portable force plate (AccuGait, Advanced Mechanical Technology, Inc., MA, USA). Ground reaction force and CoP data were collected and processed at 200 Hz using the force plate manufacturer's software (AMTINetForce 3.5.3, Advanced Mechanical Technology, Inc., MA, USA). Data were filtered with a Butterworth low pass filter with a cut off frequency of 5 Hz, down sampled to 100 Hz, and processed using custom-made functions written in Matlab (R2015b, TheMathWorks Inc., USA) to extract the variables of interest from each trial. The same standardised leg pressing warm-up based on 1-RM values obtained during the second familiarisation session, and incremental power test described in Chapter 3 were undertaken. Following five minutes of seated recovery, participants completed the assessment of plantar flexor neural drive by the twitch interpolation technique as per Chapter 3. However, no potentiated control twitch was used, with Tc instead being taken as the maximum force evoked during resting twitches.

4.2.3 Intervention

A minimisation strategy was used to assign participants to study groups to limit between group differences in mean age, BMI, and habitual steps/day size (Altman and Bland 2005). Both groups undertook 14 days of step-reduction, limiting daily walking to 1,500 steps per day. The exercise snacking group (SR+ES) undertook thrice daily bouts of the exercise snacking regime described in Chapter 3, whilst the other group undertook step-reduction only (SR). A portable pedometer was worn by participants at all times throughout the day (excluding sleep and bathing) during the step-reduction

period. Participants recorded daily step-count in a provided log book, and the pedometer automatically recorded daily step count, resetting at midnight. Participants in the SR+ES group recorded pedometer displayed steps immediately before and after each ES bout, and could remove steps added by ES bouts from the overall total number of steps for that day. Exercise snacking bouts were performed once in the morning, once around mid-day, and once in the evening, with number of sit-to-stands performed in each ES bout recorded. An advice sheet was provided to suggest strategies to reduce daily stepping. Participants also wore a SenseWear physical activity monitor armband for the first seven days of step-reduction, and recorded dietary intake on the 1st, 7th and 13th days of the step-reduction intervention using a provided dietary record sheet.

4.2.4 Retraining

Retraining consisted of six resistance training sessions across two weeks, commencing three days after the post-step-reduction trial, with sessions separated by at least 48 hours arranged at the participant's convenience. All exercise sessions were fully supervised, and commenced with the standardised warm-up on the Keiser leg press as performed during the familiarisation and testing sessions, followed by stretching at the participant's discretion. Leg press was performed with exercise intensity based on resistance of the last repetition completed during the incremental power test in the post-intervention trial. Leg extension was performed at intensities based on a 1-RM test performed during the first retraining session. Leg exercises were followed by upper body resistance exercises, alternating between two sessions (A and B) (see Table 2). Repetitions were performed at a cadence of 1:1 for concentric-eccentric time, at a comfortable participant-selected pace such that each full repetition was completed in less than five seconds. Rest intervals between sets and between exercises were 90 to 120 seconds at the participant's discretion.

Table 4.2. Retraining session content and progression

Exercise		Sessions 1 & 2			Session 3 – 6		
Session A	Session B	Sets	Reps	Load	Sets	Reps	Load
Seated leg press		3	8	60% 1-RM	4	8	75% 1-RM
Seated leg extension		1-RM test + 2 sets					
DB calf raise	DB lat raise	2	10	70% 10-RM	3	10	80% 10-RM
DB curl & press	RB seated row						

Abbreviations: DB; dumbbells, RB; elastic resistance band, Lat; lateral plane of motion, Reps; repetitions, 1-RM; maximum load at which a single repetition can be completed with correct technique, 10-RM; maximum load at which 10 repetitions can be completed with correct technique.

4.2.5 Post-RT assessment

Three days after the final retraining session, participants attended the laboratory in the morning, following a 10 hour overnight fast. Anthropometric and scanning measures were performed using the same methodology as described previously, and after which, participants were provided with a breakfast meal of their choice, with no caffeinated drinks permitted. Muscle function and balance assessments were undertaken using the same methodology as described previously; that is the measures of postural sway during standing, the leg press incremental power test, and plantar flexion twitch interpolation test.

4.2.6 Sample Size

The study was originally intended to be powered to detect the protective effect of the ES regime against loss of leg muscle power during 14 days of step-reduction. Previously, 14 days of step-reduction resulted in a decrease of 3.9 (2.4) % in lower limb muscle mass (Breen *et al.* 2013). Thus, with measures of muscle power expected to decline at a similar or faster rate, using G*Power 3 (Faul *et al.* 2007) seven participants per group was estimated as the required sample size to observe changes in leg muscle strength (95% power and 5% alpha). Without data from the study of Devries *et al.* (2015) exploring unilateral low-load resistance exercise during step-reduction, or Chapter 3 available at time of conception of the present study, it was difficult to predict the extent of the protective effect likely to be provided by the exercise snacking. Therefore, the intended sample size was rounded to 10 participants per group, to account for participant drop out and the potentially more subtle changes that might be observed in the secondary measures. However, significant challenges were encountered during the recruitment process for the present study, see Figure 4.2. for recruitment flow diagram, rendering the sample size restricting the study to a feasibility study.

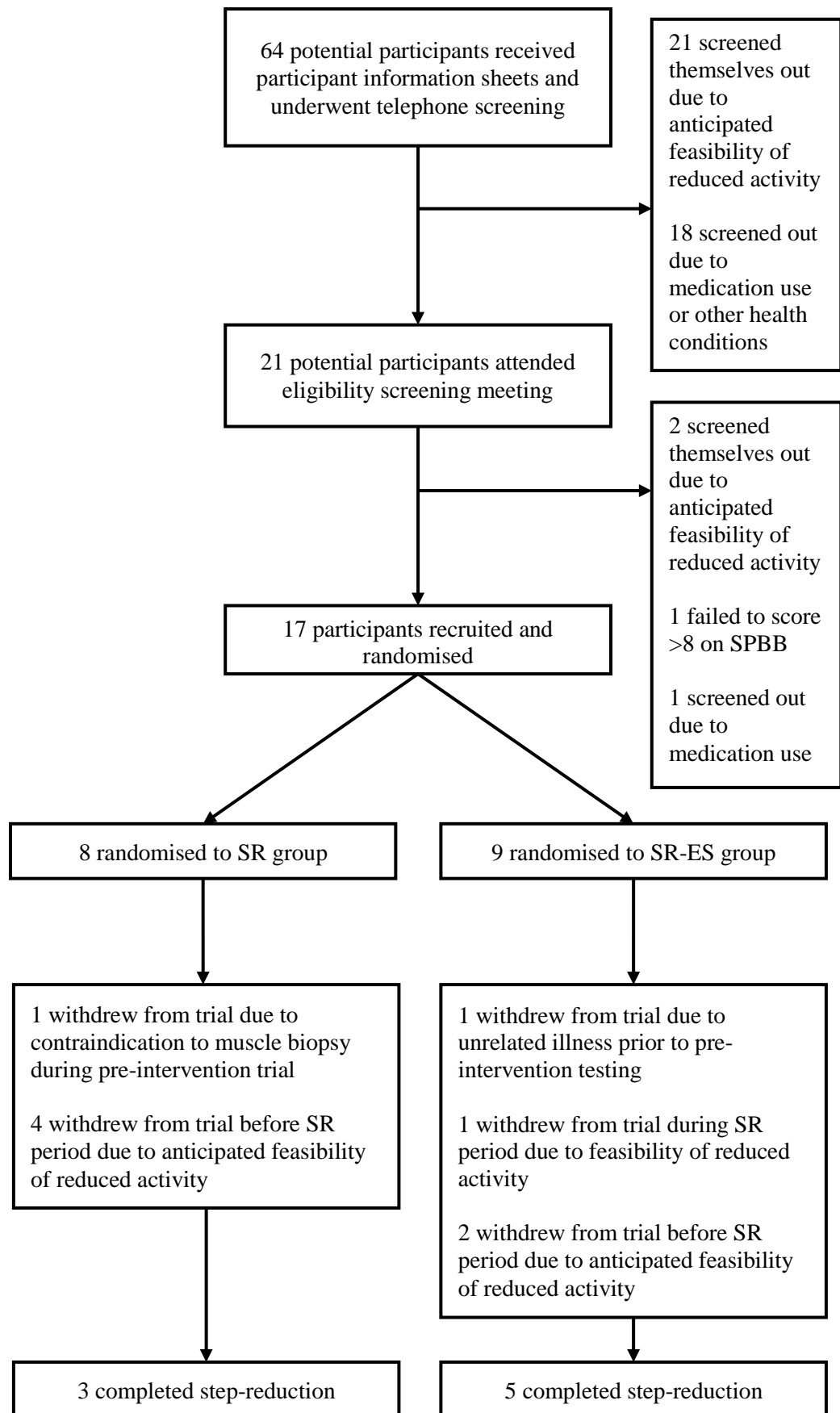


Figure 4.2. Flow diagram of participant recruitment and drop-out.

4.2.7 Statistical analyses

Due to the limited sample size completing the study, Hedges *g* effect sizes of the difference in change scores from pre-to-post-intervention, and post-step-reduction-to-post-retraining assessment was used to infer magnitude of any differences in means between groups (Hedges 1982). For variables where data were pooled for both groups to demonstrate effect of time, such as daily step-count, Hedges *g* effect size was also used to compare means of all participants from pre-to-post-intervention. Effect sizes were classified according to (Cohen 1988) with small as 0.2, moderate as 0.5, and large as 0.8. Data are presented as mean (standard deviation), and analysed using Microsoft® Excel® 2013.

4.3 Results

4.3.1 Step-reduction, physical activity, and diet

Pooling data from both groups, daily step-count reduced from 8229 (3850) steps/day at baseline to 1021 (188) steps/day during the step-reduction period, with a large effect of time ($g = 1.73$). The SR group reduced daily step count from 7455 (2061) to 1014 (192) steps/day, and after removing steps added to the pedometer count during ES bouts in the SR+ES group, steps/day reduced from 8694 (4806) to 1025 (209) steps/day (Table 4.3). The effect size of change scores between groups was small ($g = 0.31$). Without removing steps recorded in ES bouts, 1456 (366) steps/day were taken by the SR+ES group. Adherence to the ES regime was 97%, with 1 (3) out of 42 snacks missed (one participant missed six bouts, one participant missed one bout, and three participants did not miss any bouts).

Comparing PAL between baseline to the first seven days of step-reduction, pooling both groups data, PAL reduced from 1.80 (0.15) to 1.38 (0.08) ($g = 2.25$). In the SR group, PAL reduced from 1.69 (0.14) to 1.33 (0.10), and in the SR+ES group PAL reduced from 1.86 (0.15) to 1.41 (0.05), without excluding any additional activity due to the ES bouts. There was a large effect size for the change scores between groups for change in PAL or $g = 0.89$ (Table 4.3).

There were very small effect sizes for differences in change scores for daily energy, carbohydrate, and fat intake between groups (Table 4.3). When data were pooled, the effect size for the change score (pre-to-post-intervention for all participants) was also small for daily energy, carbohydrate, protein, and fat, intake. Pooling dietary data from both groups, for the three days of baseline dietary records, energy intake was 27 (8) kcal/kg/day, and for the step-reduction was 26 (6) kcal/kg/day (effect size for change over time of both groups; $g = 0.10$). Carbohydrate intake was 3.18 (1.19) g/kg/day pre-step-reduction, and 2.91 (0.79) g/kg/day during step-reduction ($g = 0.17$). Fat intake was 0.95 (0.29) and 0.97 (0.33) g/kg/day pre- and during step-reduction respectively ($g = 0.05$). There was a very large effect size for the difference in change scores for protein intake from pre- to during step-reduction between groups ($g = 1.14$). The SR group pre-step-reduction protein intake was 1.17 (0.33) g/kg/day compared to 1.09 (0.28) g/kg/day during, and the SR+ES protein intake was 0.91 (0.22) g/kg/day pre-, and 1.01 (0.20) g/kg/day during step-reduction (Table 4.3).

Table 4.3. Summary data of dietary intake from three-day diet records pre-step-reduction and during the step-reduction

	Group	Pre	During	% Δ	<i>g</i>
Steps/day	SR	7455 (2061)	1014 (192)	-86%	0.31
	SR+ES	8694 (4806)	1025 (209)	-86%	
PAL (7-days)	SR	1.69 (0.14)	1.33 (0.10)	-21%	0.89
	SR+ES	1.86 (0.13)	1.41 (0.05)	-24%	
Energy intake (kcal/kg/day)	SR	28 (8)	28 (4)	-2%	0.12
	SR+ES	26 (8)	25 (8)	-5%	
CHO intake (g/kg/day)	SR	3.31 (0.88)	3.07 (0.52)	-7%	0.02
	SR+ES	3.10 (1.44)	2.83 (0.97)	-9%	
PRO intake (g/kg/day)	SR	1.17 (0.33)	1.09 (0.28)	-7%	1.14
	SR+ES	0.91 (0.22)	1.01 (0.20)	11%	
Fat intake (g/kg/day)	SR	1.12 (0.37)	1.18 (0.34)	5%	0.23
	SR+ES	0.85 (0.21)	0.85 (0.28)	0%	

Abbreviations: SR; Step-reduction only group ($n = 3$), SR+ES; Step-reduction with exercise snacking group ($n = 5$), PAL; physical activity level as a ratio of total energy expenditure to basal metabolic rate, CHO; carbohydrates, PRO; protein. Data presented as mean (SD), % Δ pre-to-post change within groups, and Hedges *g* effect size of difference in changed scores between groups. *NB.* Steps/day were recorded for seven days of free living pre-intervention, and for all fourteen days during the step-reduction period.

4.3.2 Leg pressing and plantar flexion measures

Leg pressing V_{\max} pre-step-reduction was 1.58 (0.33) m/s and post-step-reduction was 1.65 (0.20) m/s in the SR group, and 1.78 (0.22) to 1.93 (0.33) m/s in the SR+ES group, with an effect size for the difference in change scores of $g = 0.33$. Post-retraining, V_{\max} was 1.76 (0.11) m/s in the SR group, and 2.02 (0.34) m/s in the SR+ES group. See Table 4.4 and Figure 4.3.

In the SR group, F_{\max} was 1319 (331) and 1198 (304) N pre- and -post-step-reduction respectively, and 1336 (262) N post-retraining. In the SR+ES group, F_{\max} was 1340 (214) and 1366 (355) N pre- and post-step-reduction, and 1328 (234) N post-retraining. There were large effect sizes of the difference in change scores between groups of $g = 0.86$ from pre-to-post-step-reduction, and $g = 1.48$ from post-SR to post-retraining. See Table 4.4 and Figure 4.4.

Leg pressing P_{\max} in the SR group was 544 (156) W pre-SR, 512 (152) W post-step-reduction, 599 (114) W post-retraining. In the SR+ES group, P_{\max} was 619 (81) W pre-step-reduction, 649 (78) W post-step-reduction, and 681 (102) W post-retraining, with a large effect size of change scores between groups pre-to-post-step-reduction of $g = 1.72$, and post-step-reduction-to-post-retraining of $g = 0.82$. See Table 4.4 and Figure 4.5.

One participant from the SR group could not perform maximal effort plantar flexion for the ITR during the pre-intervention trial due to muscle cramps, and another participant from the SR group could not achieve 85% of MVF in the ITR trials during the post-intervention trial. As such, for the SR group, MVF plantar flexion is reported for two participants, and no data is reported for ITR. In the SR group, MVF was 710 (514) N pre-step-reduction, 599 (440) N post-step-reduction, and 761 (558) post-retraining. In the SR+ES group, pre-step-reduction MVF was 558 (103) N, post-step-reduction was 585 (123) N, and post-retraining was 585 (92) N. Comparing change scores between groups, there was very large effect sizes of $g = 2.68$ and $g = 1.84$ for pre-to-post-step-reduction and post-step-reduction-to-post-retraining. The ITR for the SR+ES group was 86 (8) pre-intervention, and 82 (13) and 82 (10) post-intervention and post-retraining respectively (Table 4.4).

Table 4.4. Summary data of muscle function measures.

	Group	Pre-SR	Post-SR	Pre-SR – Post-SR % Δ	g	Post-RT	Post-SR – Post-RT % Δ	g
V_{max} (m/s)	SR	1.58 (0.33)	1.65 (0.20)	4%	0.33	1.76 (0.11)	7%	0.13
	SR+ES	1.78 (0.22)	1.93 (0.33)	8%		2.02 (0.34)	5%	
F_{max} (N)	SR	1319 (331)	1198 (304)	-9%	0.86	1336 (262)	12%	1.48
	SR+ES	1340 (214)	1366 (355)	2%		1328 (234)	-3%	
P_{max} (W)	SR	544 (156)	512 (152)	-6%	1.72	599 (114)	17%	0.82
	SR+ES	619 (81)	649 (78)	5%		681 (102)	5%	
PF MVF (N)	SR ($n=2$)	710 (514)	599 (440)	-16%	2.68	761 (558)	27%	1.84
	SR+ES	558 (103)	585 (123)	5%		584 (92)	0%	
PF ITR (%)	SR	-	-	-	-	-	-	-
	SR+ES	86 (8)	82 (13)	-4%		82 (10)	-1%	

Abbreviations: SR; Step-reduction ($n = 3$ unless otherwise stated), SR+ES; Step-reduction with exercise snacking group ($n = 5$), RT; Retraining, V_{max}; extrapolated maximum leg pressing velocity, F_{max}; extrapolated maximum leg pressing force, P_{max}; interpolated maximum leg pressing power, PF; plantar flexion, MVF; maximum voluntary force, ITR; interpolated twitch ratio. Data presented as mean (SD), % Δ ; percentage change between time points, and g ; Hedges g effect size of difference in change scores between groups.

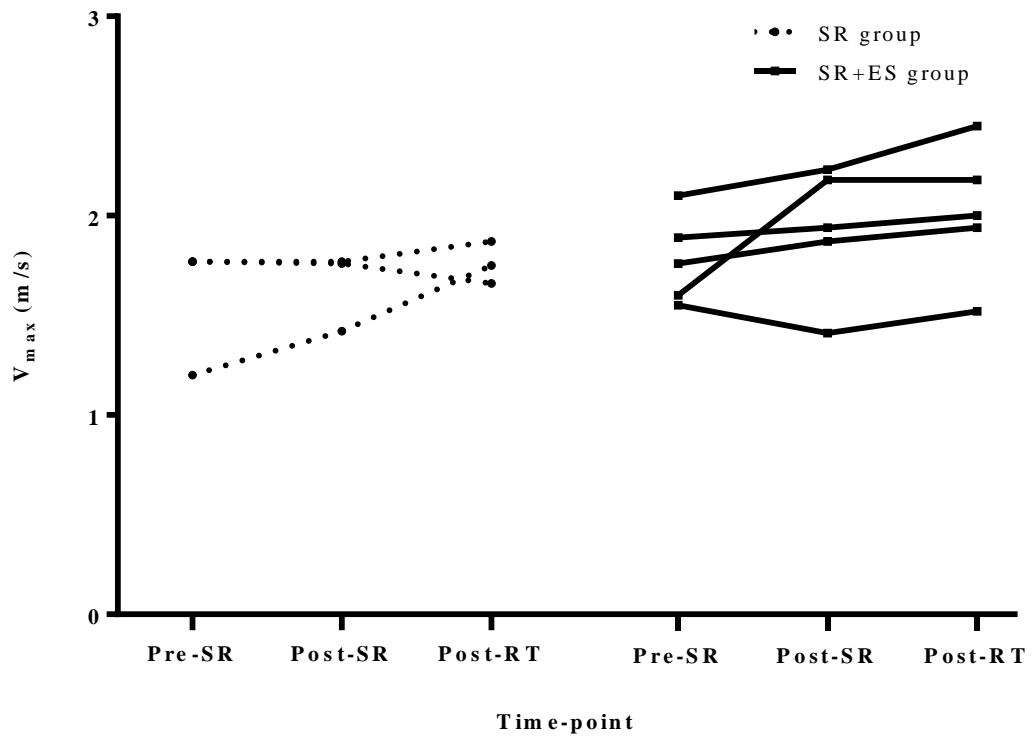


Figure 4.3. Individual changes in extrapolated leg pressing peak velocity (V_{max}) from pre-to-post-step-reduction (SR), and post-step-reduction-to-post-retraining (RT), with one group undertaking thrice daily exercise snacking during step-reduction (SR+ES).

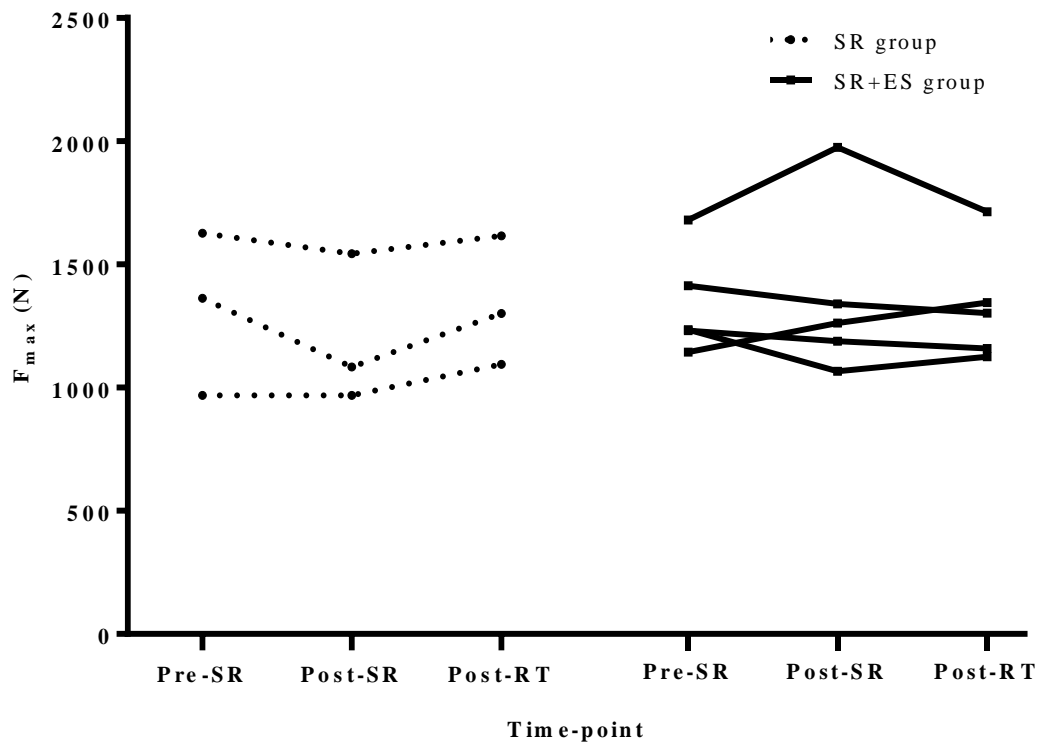


Figure 4.4. Individual changes in extrapolated leg pressing peak force (F_{max}) from pre-to-post-step-reduction (SR), and post-SR-to-post-retraining (RT), with one group undertaking thrice daily exercise snacking during step-reduction (SR+ES).

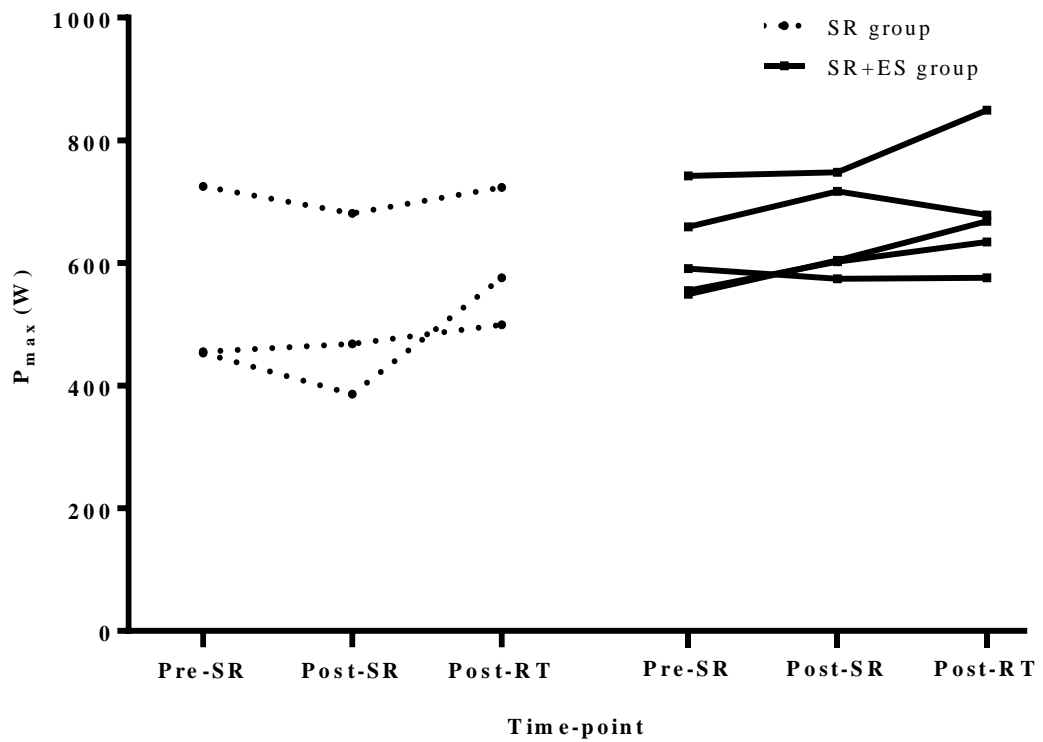


Figure 4.5. Individual changes in interpolated leg pressing peak power (P_{max}) from pre-to-post-step-reduction (SR), and post-SR-to-post-retraining (RT), with one group undertaking thrice daily exercise snacking during step-reduction (SR+ES).

4.3.3 Postural sway during quiet standing

Summary of postural sway data when eyes open and eyes closed conditions are pooled, for trials with feet 15 cm apart and feet together, respectively, are displayed in Table 4.5. With feet 15 cm apart, there were moderate effect sizes for differences in change scores between groups for CoP mean speed from pre-to-post-step-reduction ($g = 0.61$), and post-step-reduction-to-post retraining ($g = 0.69$). In the SR group, CoP mean speed was 12.4 (2.2) mm/s pre-step-reduction, 9.6 (2.6) mm/s post-step-reduction, and 10.8 (3.5) mm/s after retraining, and in the SR+ES group were 12.6 (4.0), 10.9 (2.3), and 11.0 (1.8) mm respectively. Effect size for change scores between groups were small for AP range when feet were positioned 15 cm apart. For the feet together condition, the effect size for the difference in change scores between groups for CoP mean speed was very small from pre-to-post-step-reduction ($g = 0.09$). Effect size for post-step-reduction-to-post-retraining differences was very large ($g = 1.12$; (18.1 (2.4) to 20.2 (1.5) mm/s in the SR group, and 20.2 (3.8) to 19.0 (4.6) mm/s in the SR+ES group). For the SR group AP range was 32.1 (7.3) mm/s pre-step-reduction, and 28.4 (5.5) mm/s post-step-reduction, and for the SR+ES group was 30.4 (6.9) mm/s and 34.3 (11.0) mm/s respectively, with a large difference in effect size for change score between groups ($g = 1.02$). In the SR group, post-retraining AP range was 41.1 (8.5) mm/s, and 30.4 (6.1) mm/s in the SR+ES group, with an effect size of $g = 2.70$ for the difference in post-step-reduction-to-post-retraining change score.

Table 4.5. Summary data of postural sway during quiet standing when eyes open and eyes closed conditions were combined.

		Pre-SR	Post-SR	Pre-SR – Post-SR % Δ	g	Post-RT	Post-SR – Post-RT % Δ	g
Feet apart								
CoP mean speed (mm/s)	SR	12.4 (2.2)	9.6 (2.6)	-23%	0.61	10.8 (3.5)	13%	0.69
	SR+ES	12.6 (4.0)	10.9 (2.3)	-14%		11.0 (1.8)	2%	
AP range (mm)	SR	17.4 (6.7)	15.2 (4.4)	-13%	0.51	14.4 (9.0)	-5%	0.40
	SR+ES	17.8 (5.8)	18.9 (6.2)	6%		16.0 (2.3)	-15%	
Feet together								
CoP mean speed (mm/s)	SR	20.4 (3.4)	18.1 (2.4)	-11%	0.09	20.2 (1.5)	11%	1.12
	SR+ES	22.1 (7.3)	20.2 (3.8)	-9%		19.0 (4.6)	-6%	
AP range (mm)	SR	32.1 (7.3)	28.4 (5.5)	-12%	1.02	41.1 (8.5)	45%	2.70
	SR+ES	30.4 (6.9)	34.3 (11.0)	13%		30.4 (6.1)	-11%	

Abbreviations: SR; Step-reduction only group ($n = 3$), SR+ES; Step-reduction with exercise snacking group ($n = 5$). CoP; centre of pressure, AP; anterior-posterior plane of motion. Data presented as mean (SD), % Δ ; percentage change between time points, and g ; Hedges g effect size of difference in change scores between groups.

Table 4.6 displays summary data of feet 15 cm apart and feet together trials, in both eyes open and eyes closed conditions. For brevity, only instances in which large effect sizes for the change score over time between groups were observed will be reported in this section. For CoP mean speed, the only large effect size for difference in change scores was in the feet together, eyes open condition, from post-step-reduction-to-post-retraining ($g = 1.60$). The SR group CoP mean speed post-step-reduction was 20.7 (2.5) mm/s, and post-retraining was 25.4 (1.1) mm/s, and in the SR+ES group CoP mean speed was 25.4 (5.6) mm/s and 23.7 (7.5) mm/s respectively. For AP range, there were large effect sizes for change score differences in the feet together, eyes open condition from pre-to-post-step-reduction ($g = 1.24$), and post-step-reduction-to-post-retraining ($g = 1.24$). In the SR group, pre-step-reduction AP range was 30.5 (14.1) mm, at post-step-reduction was 25.0 (2.2) mm, and post-retraining was 35.0 (16.0) mm, and in the SR+ES group, AP ranges were 24.3 (4.3), 34.4 (13.2), and 29.5 (5.6) mm respectively. For the feet together trials with eyes closed, there was a very large effect size for the difference in effect size from post-step-reduction-to-post-retraining; (31.7 (8.8) to 47.2 (3.1) mm in the SR group, and 34.1 (10.6) to 31.3 (9.0) mm in the SR+ES group; $g = 2.23$).

Comparing change postural sway in eyes open compared to eyes closed conditions over step-reduction period (i.e. assessing changes in vestibular balance), for trials with feet 15 cm the effect size between groups was moderate effect small effect sizes for CoP mean speed and AP range ($g = 0.41$ and $g = 0.36$, respectively). For trials with feet together, the between group effect size for difference in CoP mean speed between eyes open and eyes closed conditions was small ($g = 0.48$), and moderate for AP range ($g = 0.68$).

Table 4.6. Summary data of postural sway during quiet standing with eyes open and eyes closed conditions separately.

		Pre-SR	Post-SR	Pre-SR – Post-SR % Δ	g	Post-RT	Post-SR – Post-RT % Δ	G
CoP mean speed (mm/s)								
Feet apart; eyes open	SR	10.9 (1.4)	9.9 (3.6)	-9%	0.03	9.5 (3.6)	-4%	0.54
	SR+ES	9.8 (2.8)	8.7 (1.9)	-11%		8.8 (1.2)	0%	
Feet apart; eyes closed	SR	14.0 (5.5)	10.8 (3.6)	-23%	0.22	12.2 (3.5)	13%	0.66
	SR+ES	15.4 (5.3)	13.0 (3.1)	-16%		13.3 (2.5)	2%	
Feet together; eyes open	SR	15.8 (3.5)	15.6 (2.4)	-1%	0.31	15.0 (3.8)	-3%	0.07
	SR+ES	14.3 (3.0)	14.9 (2.9)	5%		14.2 (1.9)	-5%	
Feet together; eyes closed	SR	25.0 (6.8)	20.7 (2.5)	-17%	0.01	25.4 (1.1)	23%	1.60
	SR+ES	29.8 (11.7)	25.4 (5.6)	-15%		23.7 (7.5)	-7%	
AP range (mm)								
Feet apart; eyes open	SR	22.6 (11.2)	15.8 (5.6)	-30%	0.73	10.6 (3.8)	-33%	0.02
	SR+ES	19.5 (8.1)	20.9 (11.5)	7%		15.8 (4.4)	-24%	
Feet apart; eyes closed	SR	12.3 (4.9)	14.7 (3.2)	20%	0.58	18.3 (14.3)	24%	0.49
	SR+ES	16.1 (4.9)	16.9 (3.4)	5%		16.2 (4.5)	-4%	
Feet together; eyes open	SR	30.5 (14.1)	25.0 (2.2)	-18%	1.24	35.0 (16.0)	40%	1.24
	SR+ES	24.3 (4.3)	34.4 (13.2)	41%		29.5 (5.6)	-14%	
Feet together; eyes closed	SR	33.6 (6.9)	31.7 (8.8)	-6%	0.05	47.2 (3.1)	49%	2.23
	SR+ES	36.5 (9.7)	34.1 (10.6)	-6%		31.3 (9.0)	-8%	

Abbreviations: SR; Step-reduction only group ($n = 3$), SR+ES; Step-reduction with exercise snacking group ($n = 5$). CoP; centre of pressure, AP; anterior-posterior plane of motion. Data presented as mean (SD), % Δ ; percentage change between time points, and g ; Hedges g effect size of difference in change scores between groups.

4.3.4 Anthropometry

In the SR group, body mass was 76.4 (11.1) kg pre-SR, and 76.5 (10.0) kg post-step-reduction, and in the SR+ES group was 77.3 (3.7) kg and 77.8 (4.0) kg pre- and post-step-reduction respectively. The effect size of difference in change scores between groups for body mass pre-to-post-step-reduction was moderate ($g = 0.64$). Post-retraining, in the SR group body mass was 76.1 (11.2) kg, and 77.7 (4.0) kg in the SR+ES group, with post-step-reduction to post-retraining change score effect size of $g = 0.32$ (Table 4.7).

4.3.5 DXA

There was a large effect size for difference in body fat % change from pre- to-post-step-reduction between groups ($g = 1.65$). Body fat % measured by DXA pre- and post-step-reduction in the SR group was 27.3 (1.6) and 27.4 (2.1) %, and in the SR+ES group were 25.2 (3.6) and 26.5 (3.6) % respectively. Post-retraining, body fat % was 28.0 (2.7) % in the SR group, and 26.0 (3.7) % in the SR+ES group, with a large between group effect size of $g = 0.91$. Figure 4.6 and Table 4.7 display that pre-intervention whole body total fat mass was 20.7 (4.2) and 19.2 (2.6) kg for the SR and SR+ES groups respectively, and 20.8 (4.1) and 20.3 (2.6) kg post-step-reduction. There was a large effect size for difference in change scores for total fat mass over the step-reduction period between groups of $g = 1.93$. There was a large effect size for between group difference in change score from post-step-reduction-to-post-retraining of $g = 0.91$. Post-retraining, fat mass was 21.2 (4.6) and 19.9 (2.6) kg in the SR and SR+ES groups respectively. Trunk fat mass also had a large effect size for difference in change scores between groups from pre-to-post-step-reduction (12.6 (6.7) to 12.5 (2.8) kg in the SR group, and 10.9 (1.8) to 11.6 (1.7) kg in the SR+ES group; $g = 2.12$). Post-retraining, trunk fat mass was 12.7 (3.0) and 11.6 (1.6) kg in the SR and SR+ES groups respectively, with a small effect size for the difference in change scores from post-step-reduction-to-post-retraining between groups ($g = 0.34$) (Table 4.7).

Table 4.7. Summary data of body mass and dual energy x-ray absorptiometry measures.

	Group	Pre-SR	Post-SR	Pre-SR – Post-SR % Δ	<i>g</i>	Post-RT	Post-SR – Post-RT % Δ	<i>g</i>
Body mass (kg)	SR	76.4 (11.1)	76.5 (10.0)	0%	0.64	76.1 (11.2)	-1%	0.32
	SR+ES	77.3 (3.7)	77.8 (4.0)	1%		77.7 (4.0)	0%	
Body fat %	SR	27.3 (1.6)	27.4 (2.1)	0%	1.65	28.0 (2.7)	2%	0.91
	SR+ES	25.2 (3.6)	26.5 (3.6)	5%		26.0 (3.7)	-2%	
Fat mass (kg)	SR	20.7 (4.2)	20.8 (4.1)	0%	1.93	21.2 (4.6)	2	0.91
	SR+ES	19.2 (2.6)	20.3 (2.6)	6%		19.9 (2.6)	-2%	
Trunk fat mass (kg)	SR	12.6 (2.8)	12.5 (2.8)	-1%	2.12	12.7 (3.0)	2%	0.34
	SR+ES	10.9 (1.8)	11.6 (1.7)	6%		11.6 (1.6)	0%	
Lean mass (kg)	SR	54.7 (7.1)	54.7 (5.9)	0%	0.36	54.0 (7.0)	-1%	0.70
	SR+ES	57.1 (4.5)	56.7 (4.9)	-1%		56.9 (4.9)	0%	
Leg lean mass (kg)	SR	18.4 (2.9)	18.3 (2.5)	0%	0.38	18.1 (3.0)	-1%	0.85
	SR+ES	19.6 (1.2)	19.8 (1.6)	1%		19.9 (1.6)	1%	

Abbreviations: SR; Step-reduction only group ($n = 3$), SR+ES; Step-reduction with exercise snacking group ($n = 5$). Data presented as mean (SD), % Δ ; percentage change between time points, and *g*; Hedges *g* effect size of difference in change scores between groups.

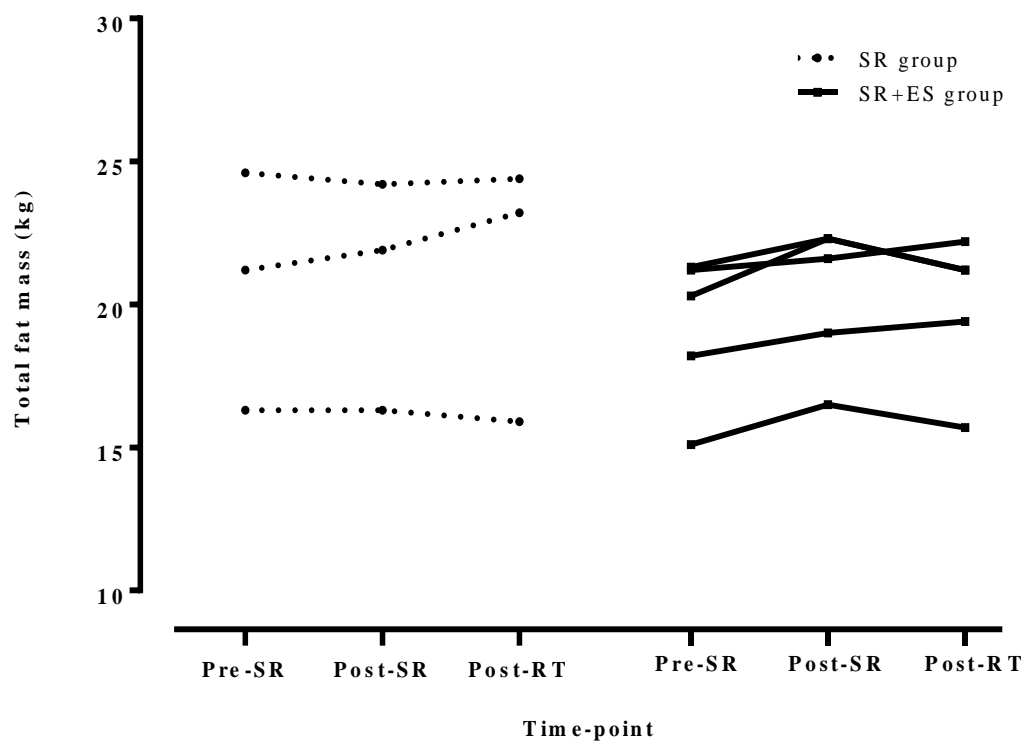


Figure 4.6. Individual changes in dual energy x-ray absorptiometry measured whole body total fat mass from pre-to-post-step-reduction (SR), and post-SR-to-post-retraining (RT), with one group undertaking thrice daily exercise snacking during step-reduction (SR+ES).

Whole body lean mass was 54.7 (7.9) kg pre-step-reduction and 54.7 (5.9) kg post-step-reduction in the SR group, and in the SR+ES group was 57.1 (4.5) kg pre-step-reduction, and 56.7 (4.9) kg post-step-reduction. There was small effect size for difference in change scores between groups from pre-to-post-step-reduction of $g = 0.36$. Post-retraining, whole body lean mass was 54.0 (7.0) kg in the SR group, and 56.9 (4.9) kg in the SR+ES group, with a moderate to large effect size for difference in post-step-reduction-to-post-retraining change score of $g = 0.70$. Leg lean mass in the SR group pre-step-reduction was 18.4 (2.9) kg, post-step-reduction was 18.3 (2.5) kg, and post-retraining was 18.1 (3.0) kg. In the SR+ES group, leg lean mass pre-step-reduction was 19.6 (1.2) kg, post-step-reduction was 19.8 (1.6) kg, and post-retraining was 19.9 (1.6) kg. The effect size for difference in leg lean mass change scores between groups from pre-to-post-step-reduction was $g = 0.38$, and for post-step-reduction-to-post-retraining was $g = 0.85$ (Figure 4.7 and Table 4.7).

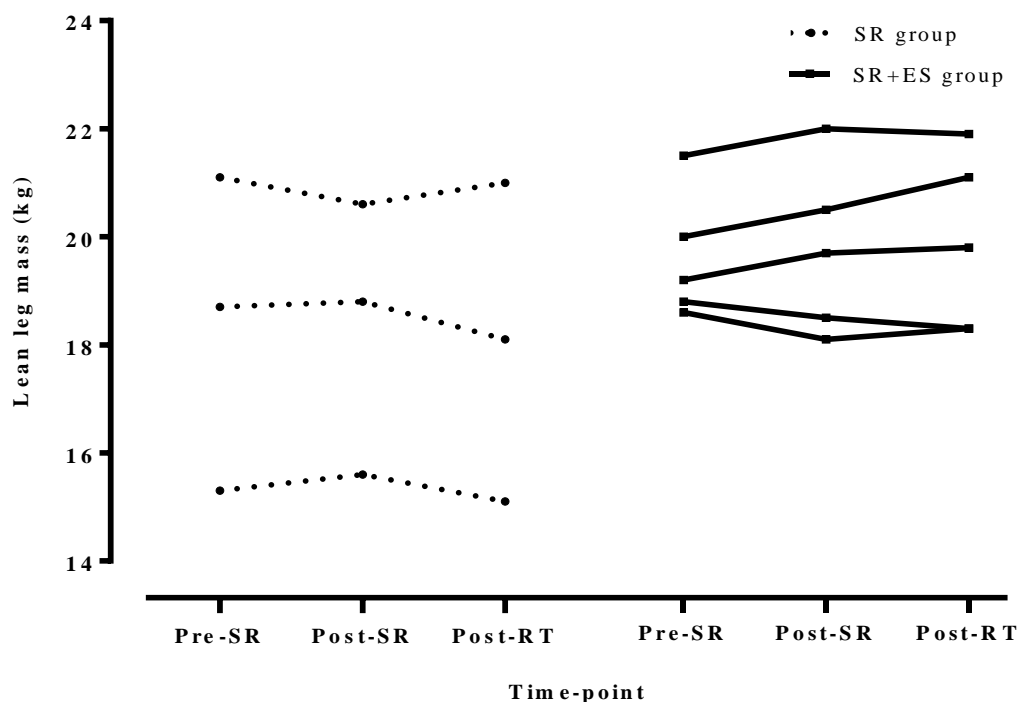


Figure 4.7. Individual changes in dual energy x-ray absorptiometry measured leg lean mass from pre-to-post-step-reduction (SR), and post-SR-to-post-retraining (RT), with one group undertaking thrice daily exercise snacking during step-reduction (SR+ES).

4.3.6 pQCT

Peripheral quantitative computed tomography data from the dominant leg is displayed in Table 4.8. Muscle cross sectional area (mCSA) at 66% of tibia length (calf) had a moderate effect size for the difference in pre-to-post-step-reduction change scores between groups of $g = 0.66$. In the SR group, pre-step-reduction calf mCSA was 8563 (2084) mm² and 8497 (1963) mm² post-step-reduction, and in the SR+ES group calf mCSA was 8205 (1476) pre-step-reduction and 8281 (1175) mm² post-step-reduction (Figure 4.8). There was a large effect size for difference in post-step-reduction-post-retraining change scores for mCSA between groups ($g = 0.80$), with mCSA of 8424 (1997) mm² in the SR group and 8256 (1170) mm² in the SR+ES group. There were small or very small effect sizes for differences in change scores of calf fat CSA and calf muscle density between groups from pre-to-post-step-reduction, and post-step-reduction-post-retraining.

At 25% of femur length, there was a very big effect size for difference in change scores of lower thigh mCSA between groups from pre-to-post-step-reduction ($g = 3.15$). In the SR group, lower thigh mCSA was 7548 (395) and 7445 (414) mm² pre- and post-step-reduction respectively, and in the SR+ES group was 7536 (975) and 7771 (971) mm² pre- and post-step-reduction respectively (Figure 4.9). From post-step-reduction-to-post-retraining there was a small to moderate effect size for the difference in change scores between groups of $g = 0.43$, with mCSA's of 7447 (367) and 7732 (920) mm² for the SR and SR+ES groups respectively. At pre- and post-step-reduction, lower thigh fat CSA in the SR group was 3711 (638) and 3750 (553) mm², and in the SR+ES group was 3799 (167) and 3891 (163) ($g = 0.61$), with post-retraining lower thigh fat CSA's of 3723 (591) and 3782 (124) mm² for SR and SR+ES groups respectively. There was a very large effect size for difference in change scores from pre-to-post-step-reduction between groups in lower thigh muscle density ($g = 1.54$), and a very small effect size for differences post-step-reduction-to-post-retraining ($g = 0.12$). In the SR group, lower thigh muscle density was 70.9 (1.7) mg/cm³ pre-step-reduction, 71.5 (1.5) mg/cm³ post-step-reduction, and 71.7 (2.2) mg/cm³ post-retraining, and in the SR+ES was 70.6 (3.2) mg/cm³ pre-step-reduction, 70.1 (3.0) mg/cm³ post-step-reduction, and 70.2 (2.2) mg/cm³ post-retraining.

At 50% femur length, the difference in change scores of mid-thigh mCSA between groups from pre-to-post-step-reduction had a very big effect size ($g = 6.97$), and from post-step-reduction-to-post-retraining had a very small effect size ($g = 0.07$). In the SR group, mid-thigh mCSA was 12959 (874) and 12819 (885) mm² at pre- and post-step-reduction respectively, and in the SR+ES group was 12966 (2216) and 13438 (2191) mm² at pre- and post-step-reduction respectively. At post-retraining, mid-thigh mCSA was 12687 (1036) mm² for the SR group, and 13294 (2072) mm² for the SR+ES group (Figure 4.10). For mid-thigh fat CSA and muscle density, there were large effect sizes for differences in change scores from pre-to-post-step-reduction ($g = 1.09$, and $g = 0.94$, respectively), with small effect sizes for difference in change scores from post-step-reduction-to-post-retraining ($g = 0.30$, and $g = 0.08$, respectively). In the SR group, mid-thigh fat CSA was 4188 (153) mm² at pre-step-reduction, 4205 (124) mm² at post-step-reduction, and 4178 (273) mm² at post-retraining, and in the SR+ES group was 3981 (502) mm² at pre-step-reduction, 4097 (511) mm² at post-step-reduction, and 4117 (528) mm² at post-retraining. At pre-step-reduction, mid-thigh muscle density was 75.5 (0.6) and 75.5 (1.0) mg/mm³ for the SR and SR+ES groups respectively, and at post-step-reduction was 75.9 (0.4) and 75.0 (1.4) mg/mm³ respectively. At post-retraining, mid-thigh muscle density was 75.4 (0.6) and 74.3 (2.2) mg/mm³.

Table 4.8. Summary data of peripheral quantitative computed tomography measures.

	Group	Pre-SR	Post-SR	Pre-SR – Post-SR % Δ	<i>g</i>	Post-RT	Post-SR – Post-RT % Δ	<i>g</i>
Calf mCSA (mm²)	SR	8563 (2084)	8497 (1963)	-1%	0.66	8424 (1997)	-1%	0.80
	SR+ES	8205 (1476)	8281 (1175)	1%		8256 (1170)	0%	
Calf fat CSA (mm²)	SR	1560 (506)	1608 (399)	3%	0.01	1558 (525)	-3%	0.32
	SR+ES	1766 (285)	1813 (362)	3%		1793 (318)	-1%	
Calf density (mg/mm³)	SR	76.5 (0.7)	76.0 (0.8)	0%	0.25	76.3 (0.9)	0%	0.33
	SR+ES	76.3 (1.9)	75.6 (2.4)	-1%		75.9 (2.8)	0%	
Lower thigh mCSA (mm²)	SR	7548 (395)	7445 (414)	-1%	3.15	7447 (367)	0%	0.43
	SR+ES	7536 (975)	7771 (971)	3%		7732 (920)	0%	
Lower thigh fat CSA (mm²)	SR	3711 (638)	3750 (553)	1%	0.61	3723 (591)	-1%	0.44
	SR+ES	3799 (167)	3891 (163)	2%		3782 (124)	-3%	
Lower thigh muscle density (mg/cm³)	SR	70.9 (1.7)	71.5 (1.5)	1%	1.54	71.7 (2.2)	0%	0.12
	SR+ES	70.6 (3.2)	70.1 (3.0)	-1%		70.2 (2.2)	0%	
Mid-thigh mCSA (mm²)	SR	12959 (874)	12819 (885)	-1%	6.97	12687 (1036)	-1%	0.07
	SR+ES	12966 (2216)	13438 (2191)	4%		13294 (2072)	-1%	
Mid-thigh fat CSA (mm²)	SR	4188 (153)	4205 (124)	0%	1.09	4178 (273)	-1%	0.30
	SR+ES	3981 (502)	4097 (511)	3%		4117 (528)	0%	
Mid-thigh muscle density (mg/cm³)	SR	75.5 (0.6)	75.9 (0.4)	1%	0.94	75.4 (0.6)	-1%	0.08
	SR+ES	75.5 (1.0)	75.0 (1.4)	-1%		74.3 (2.2)	-1%	

Abbreviations: SR; Step-reduction only group, SR+ES; Step-reduction with exercise snacking group, mCSA; muscle cross sectional area, fat CSA; subcutaneous fat cross sectional area. Data presented as mean (SD), % Δ ; percentage change between time points, and *g*; Hedges *g* effect size of difference in change scores between groups. *NB* Calf at scanned at 66% tibia length, lower thigh scanned at 25% and mid-thigh scanned 50% femur length.

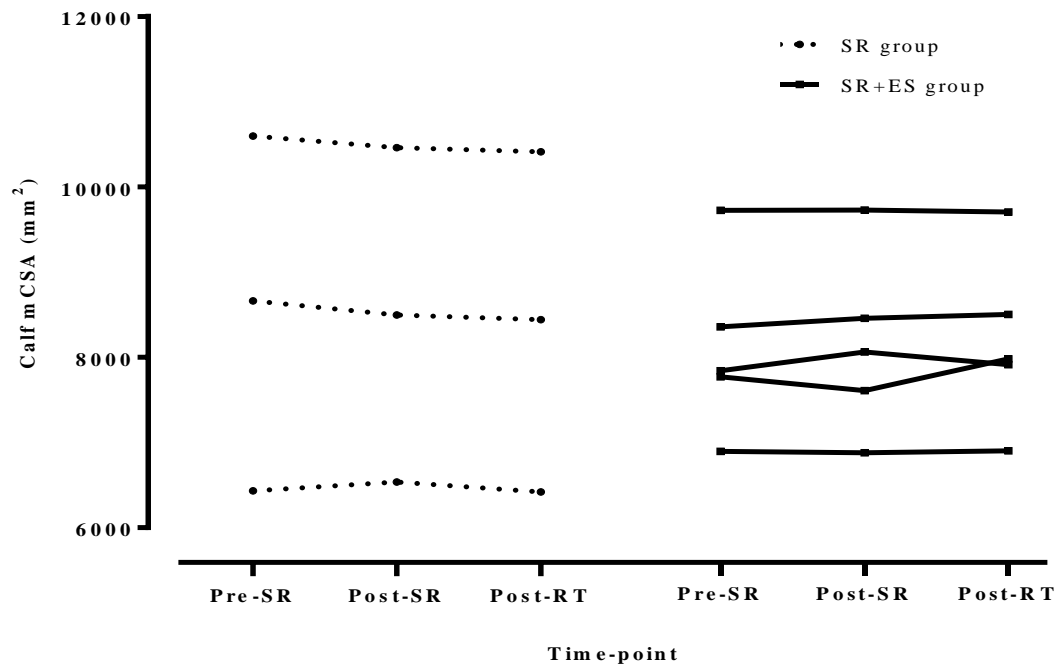


Figure 4.8. Individual changes in pQCT measured muscle cross sectional area of the calf muscle measured at 66% of tibia length distally, from pre-to-post-step-reduction (SR), and post-SR-to-post-retraining (RT), with one group undertaking thrice daily exercise snacking during step-reduction (SR+ES).

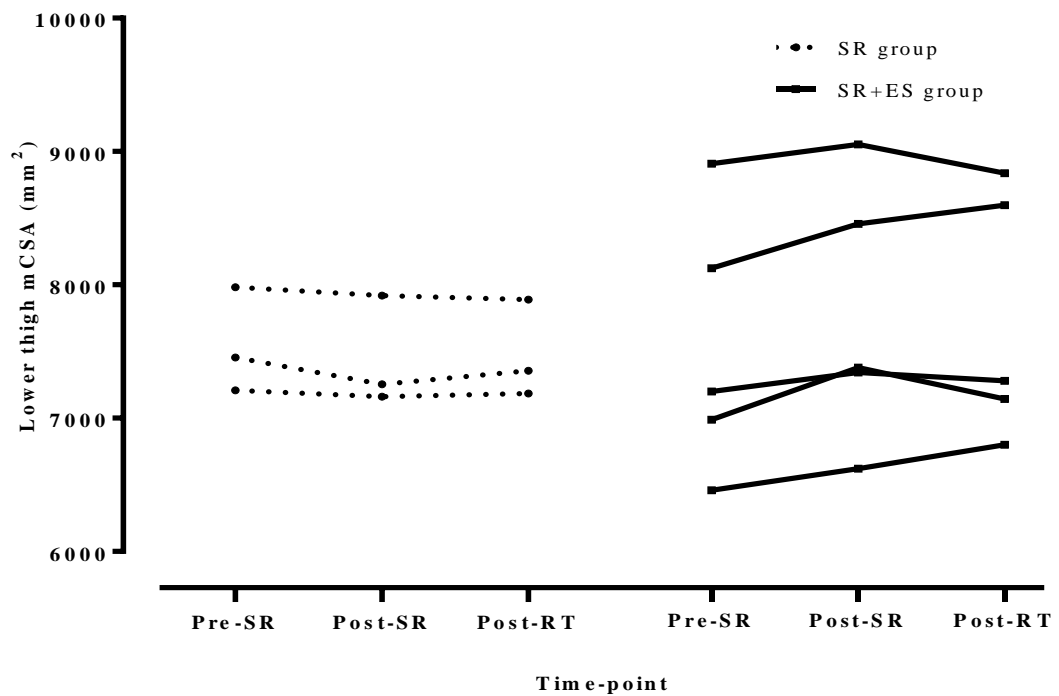


Figure 4.9. Individual changes in pQCT measured muscle cross sectional area of the lower thigh muscle measured at 25% of femur length distally, from pre-to-post-step-reduction (SR), and post-SR-to-post-retraining (RT), with one group undertaking thrice daily exercise snacking during step-reduction (SR+ES).

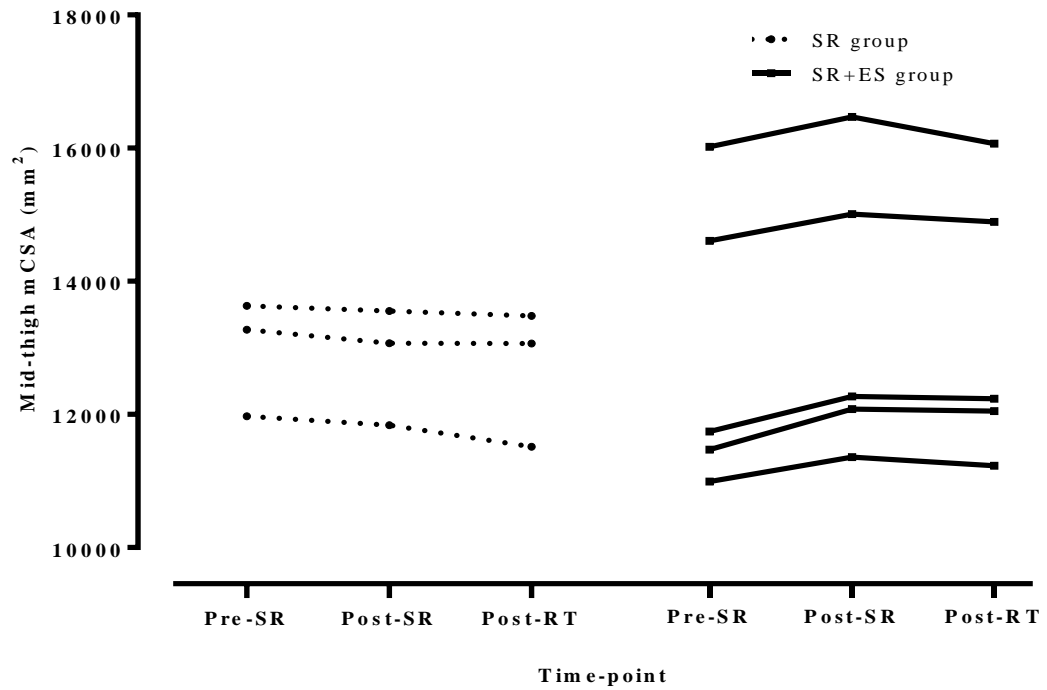


Figure 4.10. Individual changes in peripheral quantitative computed tomography measured muscle cross sectional area of the mid-thigh muscle measured at 50% of femur length distally, from pre-to-post-step-reduction (SR), and post-SR-to-post-retraining (RT), with one group undertaking thrice daily exercise snacking during step-reduction (SR+ES).

4.4 Discussion

This study aimed to establish whether exercise snacking three times a day during two weeks of step-reduction attenuated loss of leg power in healthy older men. The impact of exercise snacking during step-reduction on secondary outcomes of muscle function (velocity, strength, neural drive, and balance) and anthropometric measures were assessed. Thereafter, the effect of a two week re-retaining programme was assessed. All interpretation of findings discussed herein should be considered with the limitation of a small sample size, such that the study should only be considered as a feasibility study. Nonetheless, it appeared that exercise snacking in fact increased leg pressing power over two weeks of step-reduction, compared to a reduction in leg press power in a control group undertaking step-reduction without any protective intervention, with a similar trend for leg pressing force. Despite not DXA measured differences in muscle mass, exercise snacking during reduced activity also protected loss of thigh muscle cross-sectional area measured by pQCT. The exercise snacking regime during step-reduction did not improve efficacy of retraining for either leg muscle function or size.

Step-reduction to <1,500 steps/day resulted in an 86 (4) % reduction in daily step-count, pooling both groups together, and a drop in PAL from 1.80 (0.15) to 1.38 (0.08). This is a comparably greater relative reduction in daily steps than the 70 % and 76% observed by McGlory *et al.* (2017) and Breen *et al.* (2013) respectively, but in line with the 82% reduction that Devries *et al.* (2015) reported using the same step-reduction model. Interestingly, whilst there was large effect size for the difference in change scores between groups in PAL, it was in fact the SR+ES group displaying a greater drop in PAL with a reduction of 24% from baseline. Given that for the SR group PAL was still 21% lower than baseline during step-reduction, this is likely to be an artefact of the SR+ES group having a very high habitual PAL pre-step-reduction, in combination with the small sample size. In any case, the baseline PAL and step-counts highlight that the participants in the present study were habitually active, particularly in comparison to those described in Chapters 2 and 3 (1.59 (0.17) measured with Actiheart, and 1.67 (0.17) measured with Sensewear, respectively), and compared to the British Heart Foundation's *Physical Activity Statistics 2015* report (Townsend *et al.* 2015). Also noteworthy is the high adherence to the exercise snacking regime with 97% of bouts completed, and with three participants not missing

any bouts, and no adverse events reported. This demonstrates that exercise snacking may at least be feasible to implement in a homebased, unsupervised setting.

The key finding from the present study was that whilst, P_{\max} was 6% lower in the SR group after step-reduction, in the SR+ES group there was 5% increase in P_{\max} assessed by an incremental leg pressing test. Moreover, F_{\max} in the SR group was 9% lower after step-reduction, and increased by 2% in the SR+ES group. This demonstrates that even 15 minutes a day of unloaded lower limb exercise may have protective benefits in preserving muscle function during periods of dramatically reduced physical activity. Devries *et al.* (2015) demonstrated that 6 low load, unilateral training sessions of leg press and leg extension, increased 1-RM in the trained leg, and prevented decline in the non-trained leg. A key difference between the methodology of the present study and that of Devries *et al.* (2015) was that the exercise snacking asked participants to perform as many repetitions as possible in one minute of five exercise, whereas Devries *et al.* (2015) asked participants to achieve fatigue for each set of the two leg exercises. Whilst this difference in overall load is an important consideration, the present study also avoided participants actually practicing the test itself (Mattocks *et al.* 2017). Beyond that, the fact that participants could perform the exercise snacking in the home, unsupervised, and without any specialised equipment, makes the exercise snacking a viable strategy to implement for older adults in situations that reduce their habitual physical activity levels for a short period of time (Thiebaud *et al.* 2014).

Over the step-reduction period, albeit with a large inter-individual variability, both groups preserved or possibly even increased, leg pressing velocity when low external load was applied, with 4% and 8% increases in V_{\max} in the SR and SR+ES group respectively. That both groups, despite different interventions, and power and force outcomes, might maintain or increase leg press velocity is puzzling. In the SR+ES group, the findings could reasonably be attributed to a training response to the exercise snacking, which is intuitively appealing given that unloaded repetitions at reasonably high contraction speeds was the cornerstone of the regime. In older women (aged 77 (6) years; SPPB score 8 (1)) undertaking similar exercise regime to exercise snacking whilst wearing an externally loaded weight vest, Keiser leg press speed at 40%-1RM increased by 23% (though not significantly ($p = 0.058$)) after 12 weeks of three training sessions a week (Bean *et al.* 2004). This study did not use the extrapolation method employed in the present study, however, this reinforces that

performing chair based exercises at maximum contraction speed likely increases leg pressing speed. The more perplexing finding is that in the SR group, V_{\max} did not change in concordance with P_{\max} and F_{\max} . There is evidence of preferential loss of type I muscle fibres in severe forms of unloading such as space flight and bed rest (Fitts *et al.* 2010). Also, increases in unloaded shortening velocity of *ex vivo* muscle fibres have been demonstrated after bed rest and space flight (Widrick *et al.* 1997; Widrick *et al.* 1999). However, implicating the same mechanisms that led to increased fibre shortening characteristics as described in the aforementioned bed rest and space flight research in the observed possibly increased V_{\max} would be something of a leap of faith. Indeed, with only three participants completing the intervention in the SR group, and the variation in data seen V_{\max} for the SR group may simply be noise in the data.

Whilst the power and force data from the leg pressing variables pre-to-post-step-reduction align with what may have been anticipated from previous literature, the changes with retraining are somewhat less clear. Whilst P_{\max} increased by 5% with retraining in the SR+ES group, there was a counterintuitive reduction of 3% in F_{\max} in the SR+ES group. It could be argued that participants may have been fatigued from previous retraining sessions, however, with three days between the final training session and the post-retraining assessment, this seems unlikely. Furthermore, this pattern wasn't observed in the SR group. In fact, the SR group increased F_{\max} by 12% over the retraining period, with an increase in P_{\max} of 17% from post-step-reduction-to-post-retraining. Whilst the change in P_{\max} is in the context of likely transient decrease in both power and force production capability immediately following step-reduction, this increase is high. In older women training three times a week with gym based leg strength exercises, Loenneke *et al.* (2017) observed non-significant increases in leg press 1-RM of approximately 14%. This is comparable with the strength gains in the SR group study despite the prior reduced activity period. Following a more extreme disuse model in older men (14 days of bed rest), following two weeks of retraining older men only recovered 8 (9) % explosive leg pressing power of the 12 (10) % lost with bed rest (Pisot *et al.* 2016). Inspecting shorter term changes, Hvid *et al.* (2014) demonstrated that seven days of active recovery, including two bouts of resistance exercise, was not sufficient to restore knee extensor isometric and dynamic strength, or rate of force development (RFD) after just four days of immobilisation in old males. However, undertaking three resistance exercise sessions

a week for four weeks immediately after two weeks of leg immobilisation restored maximal isometric and dynamic force, although RFD remained slightly depressed compared to pre-immobilisation capability (Hvid *et al.* 2010). It is possible that even the limited steps per day permitted in the present study protected the pronounced losses in power reported in rest and immobilisation studies, and may improve recovery speed.

A possible consideration for divergent responses to the retraining between groups in the present study could be the relative intensities at which leg press exercise took place. Resistance training load was calculated based on the maximum resistance against which a repetition was completed during incremental power test in the post-step-reduction main trial. Interpolated P_{max} and extrapolated F_{max} would be increased by generating more power at each prescribed leg press resistance of the incremental power test, but without the need to actually complete a repetition at a higher than previously achieved 1-RM on the leg press. As such, participants that increased force and power producing capability, but didn't achieve a new 1-RM on the leg press, may have then been prescribed training loads at a relatively lower exercise intensity than they would have had they repeated a 1-RM test with the option to increase resistance by small increments. In any case, the large increase in P_{max} with retraining in the SR group highlights that healthy older adults are capable of regaining losses in leg strength and power immediately after a period of reduced activity without any other protective intervention. This is important as it represents an opportunity to potentially decrease the time spent in a less functionally able state after a period of reduced activity (Shearer and Guthrie 2013).

In an attempt to provide an explanatory mechanism for changes in leg muscle function, assessment of neural drive by twitch interpolation of plantar flexion was undertaken. In the SR group, ITR data were available for only one participant, and MVF data available for two participants. For the two SR participants, isometric plantar flexion MVF decreased by 16% from pre-to-post-step-reduction, and increased by 27% from post-step-reduction with retraining. This is in line with what may be expected considering the aforementioned changes in leg pressing measures. Equally congruent with the leg press data for the SR+ES group, there was an increase in MVF of 5% during step-reduction, with no further change following retraining. However, in the SR+ES group, there was a 4 (10) % drop in ITR with step-reduction, despite the apparent increase in isometric strength. Whilst not always demonstrated empirically (Horstman *et al.* 2012), a reduced neural drive capacity would be expected to

following unloading which would likely decrease ability to generate maximum torque (Gondin *et al.* 2004). As such, the findings of the present study are not physiologically rational, so can most likely be put down to a combination methodological inaccuracy and small sample size. Moreover, inspecting the effect of time on ITR in the SR+ES alone, with only a small effect size ($g = 0.22$), this apparent 4% reduction in ITR within a small sample size likely to be noise within the measurement.

Previous research has demonstrated that lower limb muscle size might be responsive to the interventions undertaken in the present study. Based on data from (Breen *et al.* 2013) and (Krogh-Madsen *et al.* 2010), observing 3.9% and 2.7% decreases in leg lean mass in old and young participants respectively after two weeks of step-reduction to <1,500 steps per day, it was anticipated that the SR group would respond similarly. However, this was not supported by the present data; there was pronounced heterogeneity in DXA estimated leg lean mass responses to the step-reduction intervention, with neither group mean changing. Furthermore, the calf muscle, a site previously not investigated specifically in the context of step-reduction and atrophy, also did not demonstrate pronounced differences in mCSA between groups, with a moderate effect size for the difference in change scores. In the SR group, mCSA of the calf decreased by 1%, and in the SR+ES group increased by 1%. With the load bearing role of the plantar flexors, bed rest research would suggest a heightened sensitivity to disuse (Trappe *et al.* 2008). Without previous studies investigating the effects of step-reduction on calf muscle size, the present data suggest that even the limited ambulation undertaken in the present study may be enough to preserve calf muscle size.

The differences in the change scores for lower and mid-thigh mCSA from pre-to-post-step-reduction were markedly more distinct between groups. At the lower thigh (25% of femur length proximally from femoral lateral epicondyle) the SR+ES group increased mCSA by 3%, compared to a 1% decrease in the SR group, with an effect size of $g = 3.15$. At the mid-thigh (50% of femur length), mCSA increased by 4% in the SR+ES group and decreased by 1% in the SR group, with an effect size of $g = 6.97$. The strength of the effect sizes may be exaggerated by the small sample size, but nonetheless it can be said with some confidence that for the thigh mCSA, exercise snacking was protective against step-reduction induced atrophy.

It should be noted that whilst a 4% increase in mid-thigh mCSA seems remarkably high, DeFreitas *et al.* (2011) has reported increases of 4.5% in mid-thigh

mCSA in two weeks of resistance training in healthy young males. It was suggested that an initial increase in mCSA observed by DeFreitas *et al.* (2011) was likely to be exercise induced oedema, as the training regime was initially associated with post-exercise muscle soreness. The threshold based analysis employed in pQCT scan analysis in this, and the present, study, cannot differentiate between hypertrophied muscle and inflammation, however the authors contend that after the first week of exercise, any increase in mCSA could reasonably be attributed to muscle growth. Damas *et al.* (2015) goes so far as to state that any observed early mCSA increase, induced resistance training, without concomitant measures of muscle oedema should not be labelled as hypertrophy. However, although further investigation is required to confirm this, it is anticipated that the exercise snacking would be unlikely to stimulate pronounced inflammatory responses, especially after two weeks to become accustomed to the regime. Another possible explanation to rationalise the large increase in mCSA could be related to glycogen loading within the muscle, as put forward by Walhin *et al.* (2013) to explain 2.6 kg increases in DXA estimated whole body lean mass following seven days of reduced activity to <4,500 steps per day with overfeeding. If glycogen loading was implicated in the increased thigh mCSA in the present study, the DXA lean mass estimates do not support this contention, as whole body lean mass did not increase. However, there were 1% decreases in muscle density at both lower and mid-thigh in the SR+ES group, so whether that reflects an increase in muscle glycogen and associated water storage would require further investigation. Though risking over interpretation of the limited data set available, it is notable that the apparent improvements in functional and anthropometric outcomes are at least for the most part compatible for the SR+ES group.

Breen *et al.* (2013) observed a significant increase in total body fat %, and trends towards increased trunk fat mass (0.33 kg increase; $p = 0.053$) measured on DXA in older adults reducing steps to <1,500 per day for two weeks. Very similar findings for the SR+ES group were observed in the present study, with DXA measures of total body fat % increasing from 25.2 (3.6) % to 26.5 (3.6) %, and a 0.62 kg (0.41) kg increase in trunk fat. However, in overweight older adults undertaking step-reduction for two-weeks (McGlory *et al.* 2017), and in the other available example of step-reduction with protective exercise in older adults (Devries *et al.* 2015), no changes in fat mass from pre-to-post step-reduction were observed. More confusingly still, there were very large effect sizes for difference in body fat % and trunk fat change

scores over the step-reduction period between groups ($g = 1.65$ and $g = 2.12$ respectively). The SR group body fat % did not change over the step-reduction period, and trunk fat apparently decreased by 0.13 (0.21) kg. As previously discussed, although the SR+ES group did reduce daily PAL by comparatively more than SR group, the SR+ES group was apparently still more active during the step-reduction period with PAL of 1.41 (0.05) compared to 1.33 (0.10) in the SR group. However, based on the food diary data, there was only a very small effect size ($g = 0.12$) for the difference in change scores in caloric intake between groups, so it is hard to understand why there would be a marked difference in change in fat mass between groups measured by DXA. Furthermore, the pQCT measures of fat CSA at the thigh are to some extent in concordance with the whole body imaging measure. There were moderate and large effect sizes for differences in lower and mid-thigh fat CSA change scores between groups, with the fat CSA increasing by 2% and 3% in lower and mid-thigh respectively for the SR+ES group, compared to an increase of 1% in the lower thigh and no change at mid-thigh in the SR group. Both groups increased calf fat CSA by 3%, with no difference in change scores between groups, although even when groups were pooled the effect size for the change over time was very small ($g = 0.10$).

The anthropometric responses to retraining are equally as difficult to explain as the functional outcomes. Following retraining, there was a large effect size for the difference in change scores for DXA estimated total body fat % and fat mass, with the SR group increasing fat mass by 2%, and the SR+ES group decreasing by 2% ($g = 0.91$). The pQCT data does not add any clarity regarding changes in adiposity, insofar as the SR group had 3% lower fat CSA at the calf, and 1% lower fat CSA at both thigh sites. Whilst site specific changes in fat CSA of the lower limb are by no means crucial outcomes, it could be expected that there would be a general agreement between total body fat mass and leg fat CSA. Conversely, there was no change in fat CSA at the mid-thigh in the SR+ES group, but a 3% reduction at the lower thigh, and 1% reduction at the calf. Again, the findings for DXA estimated lean leg mass were perhaps not as anticipated, with the SR group apparently losing 1% and the SR+ES group gaining 1% from post-step-reduction. The pQCT data again were generally suggestive of very minimal changes in mCSA at both the calf and thigh measurement sites, with no changes of more than 1% in any direction across all sites. With no comparative data from previous literature on recovery from step-reduction, and without any physical activity monitoring or diet records, any explanation as to what

may have caused these unexpected responses would be unsupported speculation. At least in the SR group it would seem that the responses of muscle function and anthropometry reflect the traditional view that with onset of resistance training, changes in strength occur prior to hypertrophy (Folland and Williams 2007a).

As well as attempting to assess changes in leg press power and force, this study also sought to explore whether balance during quiet standing was also affected by step-reduction and exercise snacking during step-reduction. Paillard and Noé (2015) present that the most reliable measure of postural sway is CoP mean speed, and in the present study there were no noteworthy differences in the change in CoP mean speed over the step-reduction period between groups. Moreover, in all the trials in which feet were positioned 15 cm apart, of which there were three repeats for each condition (eyes open and eyes closed), there were no large effect sizes recorded for difference in change scores between groups over the step-reduction period. Melzer *et al.* (2004) highlight the importance of assessing postural sway with feet together to reduce the base of support size when considering fall risk, particularly in older adults. In the feet together trials in the present study, both for combining eyes open and eyes closed data, and when taking the eyes open data alone, there were large effect sizes for the difference in change scores between groups for AP range ($g = 1.02$ and $g = 1.24$, respectively). Unexpectedly the data suggest that it was in fact the SR+ES group whose postural sway was worsened during the step-reduction period, despite exercise snacking being protective against muscle power and force loss. Whilst this is counterintuitive, it should be considered that the feet together trials were single trials without repeats, so may be more prone to variability. Nonetheless, Kouzaki *et al.* (2007) have shown that 20 days of bed rest in healthy young males increased CoP mean speed with 15 cm apart, both with eyes open and eyes closed. Moreover, one group in the aforementioned study undertook twice daily leg pressing and calf extension exercise on 16 of the 20 days of bed rest, maintaining plantar flexor muscle volume, but exercise inferred no protective effect against increased postural sway during quiet standing. Again it may be that the limited amount of walking permitted in the present study was sufficient to maintain postural stability in quiet standing, or the sample size was insufficient to detect any changes. From the limited data in the present study, it also appeared that there was no effect on the vestibular component of balance of either intervention. There were only small effect sizes (CoP mean speed in all trials, and AP range with feet apart) or moderate effect sizes (AP range with feet

together) for the difference in change scores in postural sway difference between eyes open versus eyes closed conditions, from pre-to-post-step-reduction between groups.

All participants were given the same instructions with regards to dietary intake, and there was only a modest change in energy intake, with small effect sizes for the difference in change scores between groups for each macronutrient intake apart from protein. Pooling both groups, daily energy intake in calories reduced by 4%, with an 8% decrease in carbohydrate intake, but a 2 % increase in fat intake. With a limited sample size and only three days of diet records at each time point, these changes in macronutrient intake are probably trivial, but clearly did not align with the reduced energy expenditure (23% reduction in PAL). With more participants there may have been merit in exploring differences in energy intake across a period of step-reduction. There was an unexpected difference in the change in daily protein intake between groups which is not easy to explain, with the SR group reducing protein intake by 7%, and the SR+ES group increasing protein intake by 11%. Again, this may be an artefact of self-report nature of data, and the data coming only from three days, although (Fyfe *et al.* 2010) indicates that three days of diet records are sufficient represent habitual dietary intake for seven days. Crucially however, protein intake in the present study of 1.0-1.1 g/kg/day was slightly lower than is suggested for preventing deconditioning during reduced activity (1.2-1.6 g/kg/day) and therefore represents a behaviour that could justifiably be targeted in older adults undergoing periods of reduced activity (Phillips *et al.* 2016; Wall and van Loon 2013). In the present study however, despite the suboptimal dietary protein content during step-reduction, there still was no marked drop in lean tissue in the SR group, and the SR+ES group in fact increased thigh mCSA.

The most obvious limitation of the present study is the limited sample size. As Figure 4.2 presents, recruitment for this study was challenging, primarily due to inherent logistical issues of limiting daily steps to <1,500 per day. Nine eligible participants completed screening and baseline physical activity assessment, yet did not complete the study. A secondary implication of participant drop out is that attempts to stratify participant allocation by minimisation (Altman and Bland 2005) were undermined when a participant was allocated to a group and subsequently dropped out after other participants had be allocated groups based on evolving group means. When attempting to analyse data from such a limited data set without an appropriate statistical analysis beyond *Hedges g* effect sizes, employing a more conservative

corrected pooled standard deviation (Hedges 1982), there is an inherent tendency to suggest that findings that fit the preconceptions and previous data are ‘suggestive’ of an effect, whereas findings that are counter-intuitive are disregarded as noise in the measurement due to a small data set. The findings of the present study will be inherently limited in applicability due to the inclusion of only healthy, non-smoking, non-obese, non-frail males, and the exclusion of more common clinical phenotypes for this age group. Moreover, the findings will hold limited generalisability to older individuals taking regular anti-inflammatory medication and to older women. A further limitation of the present study is that these findings cannot inform on recovery from reduced activity when simply returning to habitual activity levels without a specific retraining regime.

Reflecting on the challenges encountered in carrying out the present study, the practical implication of step-count restriction in healthy, independent older adults, was a substantial obstacle that dissuaded potential participants from entering the study. This should be carefully considered when attempting to undertake future work in this area. Whilst bed rest studies can be relatively easily contextualised for potential participants, a common occurrence in the present study was that it wasn’t until potential participants wore a pedometer and objectively quantified their habitual activity did they appreciate the restriction that step-reduction would impose. Future work in this area might look to provide more support to participants during the step-reduction period, such as provision of mobility scooters or personal assistance with day-to-day tasks requiring ambulation such as grocery shopping or errand running. This was beyond the available resources for the present study, as were direct financial incentives. Alternatively, investigating shorter durations of step-reduction, or less severe step-reduction might improve participant recruitment.

An interesting question that remains to be answered is whether a relatively greater decrease in physical activity leads to greater loss of muscle function or mass, or whether actually there is a protective effect of being more active. Thereafter, if such a protective effect did exist, whether this could be optimised through focussed exercise programmes may provide a protective strategy to attenuate loss of muscle function and mass in reduced activity scenarios when exercise snacking could not be undertaken. Whilst there were no obvious effects of step-reduction postural sway during quiet standing, it may be that more dynamic measures of balance could be compromised, such as ability to recover from a trip whilst walking. There are also

more questions specifically around the exercise snacking regime itself, firstly regarding whether three bouts per day is required for a protective effect, and if a different or more varied set of exercises would increase efficacy. Thereafter, exploring whether the exercise snacking is suitable for less healthy or functionally compromised older adults, or even whether it may be suitable for a clinical setting where reduced activity is may take the form of a hospital stay.

In conclusion, in a very limited sample size, this study did not replicate the previously observed decreases in lean leg mass in older adults undertaking step-reduction without protective exercise interventions by Breen *et al.* (2013), rather it more closely aligned with data from McGlory *et al.* (2017) observing no loss of muscle mass with step-reduction for two-weeks. The present study is the first to suggest that leg muscle power and strength in dynamic movements may be compromised by step-reduction without protective intervention. However, two weeks of resistance training were sufficient to restore lost leg muscle function after step-reduction. The key finding from the present study was that exercise snacking three times a day during two-weeks of exercise snacking may have potential to not only attenuate loss of leg function and muscle mass, but may increase leg press power and thigh muscle cross sectional area.

Chapter 5: General Discussion

5.1 Overview

The overall aim of this thesis was to examine the role of physical activity and exercise in preserving muscle function and mass with ageing in healthy adults. In order to do this, a cross-sectional observational study, and two intervention studies were conducted.

Specifically, Chapter 2 sought to compare habitual physical activity and leg muscle power, strength, and velocity between healthy, free-living older adults to younger adults, and explore the relationship between physical activity and leg muscle function characteristics. Chapter 3 then examined the effect of a short term, homebased exercise snacking programme, supported by a nutritional supplement, on muscle function and size in healthy, non-exercising older adults. Chapter 4 attempted to implement that exercise snacking regime as a means to attenuate loss of muscle function and mass induced by two weeks of step-reduction in healthy older men. A secondary objective of Chapter 4 was to explore the effect of two weeks of retraining with resistance exercise after step-reduction, and whether this was altered by exercise snacking during step-reduction.

5.2 Summary of findings

Chapter 2

- Older adults had comparatively lower leg pressing power, force, and velocity producing capability than young adults, both in absolute terms and relative to thigh muscle mass, despite maintaining habitually active lifestyles.
- No objectively measured metric of physical activity was associated with any measure of muscle function when assessed by pneumatic leg press dynamometry.

Chapter 3

- Two bouts of exercise snacking a day for 28 days, consisting of five leg exercises, with as many repetitions as possible completed in one minute per exercise, accompanied by nutritional supplement of yogurt at breakfast, increased the maximum number of sit-to-stands from a chair in 60 seconds by 31%.
- Exercise snacking induced non-significant increases in leg pressing interpolated maximum power, and power at 40% of 1-RM, but with large effect sizes for differences in change scores compared to a control group not undertaking any exercise.
- There was a large effect size for the difference in change scores between groups for thigh muscle cross sectional area, with a non-significant trend towards an increase in thigh muscle cross sectional area of 2% after 28 days of exercise snacking.
- Exercise snacking did not result in improved isometric plantar flexion strength, postural sway during quiet standing, or improved six minute walk test time compared to controls.
- Exercise snacking did not result in changes in total body fat %, total body or leg lean mass, or calf muscle cross sectional area, compared to controls, although there was a significant decrease in calf fat cross sectional area with a 28 days of exercise snacking.

Chapter 4

- All findings mentioned below should be considered in the context of very small sample sizes due to high participant dropout (three participants completed two weeks of step-reduction, and five completed step-reduction with exercise snacking three times a day).
- Older men undertaking two weeks of step-reduction to <1,500 steps per day without intervention lost muscle power and strength in leg pressing, but with no concomitant loss of lean mass or muscle cross sectional area.
- Exercise snacking three times per day during step-reduction resulted in a 2% increase in maximum force, and 5% increase in maximum power in leg press dynamometry, with large effect sizes between groups for change scores.
- Exercise snacking during step-reduction also increased lower and mid-thigh muscle cross sectional area by 3% and 4% respectively, again with very large effect sizes for differences in change scores.
- The efficacy of two weeks of retraining with resistance exercise was not improved by exercise snacking during step-reduction.
- Step-reduction, with or without exercise snacking, seemed not to affect postural sway during quiet standing.

5.3 Discussion

5.3.1 Ageing, muscle, and physical activity

The somewhat obvious finding of Chapter 2 was that older individuals were not as strong or powerful as young individuals tested by pneumatic leg press. Older individuals had less muscle mass, extrapolated from 19% lower thigh muscle thickness (Franchi *et al.* 2017), so the 33% lower extrapolated maximum force and 51% lower interpolated maximum power were perhaps to be expected (Fukunaga *et al.* 2001). Indeed, habitual PAL for the older individuals in Chapter 2 was also significantly lower in absolute terms than the younger individuals in that study (1.59 (0.17) vs 1.74 (0.23) respectively). However, when comparing MVPA between the groups based on age specific cut offs for METs, the older group undertook significantly more MVPA than the younger group. Despite the older group engaging in twice the MVPA time as the younger group, F_{\max} was 16% lower and P_{\max} 38% lower when considered per unit of *vastus lateralis* muscle thickness on a pneumatic leg press. Whilst this is not a particularly novel finding, it does reinforce that age is associated with not only lower muscle mass, but also a compromised ability for the remaining muscle to produce force. Importantly, the relative differences between the age groups for power compared to force also supports that the ability to produce force quickly deteriorates with age. As highlighted by Bean *et al.* (2002), power is the crucial muscle function characteristic determining physical performance when older adults become more limited in mobility.

It is intuitively appealing to presume that in older adults, higher habitual physical activity levels would beget higher muscle power and force producing capability, both by virtue of preserved muscle mass and maintained muscle quality in terms of specific force. Granted, this correlation does not infer causality, but at a population level this is broadly the case, taking into account the range of very sedentary and very active individuals (Paterson and Warburton 2010). In generally healthy, active older adults, the relationship seems to be less clear. In Chapter 2, there were no associations between physical activity, considered by any objectively measured index, and muscle function, be that in absolute terms or relative to muscle size. Whilst habitual physical activity was represented by only one week of monitoring, and the findings of Togo *et al.* (2008) suggest that more sporadic periods of monitoring are needed to reliably predict annual physical activity, these data suggest

that physical activity *per se* does not predict maximum force and power producing capability in active older adults. For context, it is important to stress that these data do not suggest physical activity is not vital for healthy ageing (McPhee *et al.* 2016). Clearly physical activity is a cornerstone of healthy of ageing (Hamer *et al.* 2013) but concerted efforts to engage in resistance type exercise may be required to maintain force and power into older age. This has been demonstrated longitudinally by Hughes *et al.* (2001) in healthy active older men, insofar as changes in physical activity were not associated changes in isokinetic knee extensor strength over 10 years. An important consideration is also that the physical activity monitoring devices used in the studies of the present thesis were not capable of recording movements that were load bearing. Indeed, the exercise snacking bouts were not identifiable in the raw data collected by the SenseWear armband device (BodyMedia, Inc., Pittsburg, PA, USA). As such, the physical activity data cannot identify those participants who were performing resistance type exercise.

Although habitual physical activity does not appear to be predictive of muscle force and power producing capabilities in healthy older adults, Chapter 4 sought to demonstrate the impact of drastically reducing physical activity for a two week period. Previously, using isometric dynamometry, neither Breen *et al.* (2013) nor McGlory *et al.* (2017) observed any loss of leg strength following two weeks of step-reduction in older adults, despite the former reporting a 4% loss of leg lean mass. The previous literature on muscle disuse, particularly in older adults and even for relatively short periods of time, suggest that at least some indices of muscle function may be compromised with step-reduction (Kortebein *et al.* 2008; Hackney and Ploutz-Snyder 2012; Coker *et al.* 2015). The present findings suggest that dynamic leg pressing force and power may be reduced by step-reduction when no protective interventions are employed. After step-reduction, F_{\max} was 9% lower and P_{\max} was 6% lower, and surprisingly, this function loss appeared to be in the absence of leg tissue loss. It is interesting that force seemed to be reduced to a greater extent than power. Neural function, for which power is considered to be indicative, would be expected to occur more rapidly than changes in mCSA, which would be considered more important for force production (Folland and Williams 2007a). However, it again must be stressed that these data come from only three participants, and as Figures 4.4 and 4.5 display, there was notable inter individual variation in the responses.

5.3.2 Short bouts of homebased exercise

Historically, studies investigating exercise during periods of unloading have been concentrated on identifying means to reduce the muscle wasting effects of microgravity during space flight, with varying degrees of success dependent of the variables in question and the interventions explored (Trappe *et al.* 2004; Rittweger *et al.* 2005; Akima *et al.* 2000). Devries *et al.* (2015) applied this concept to a step-reduction model with low load unilateral leg exercise, and demonstrated that exercise during step-reduction not only prevented loss of strength, but in fact improved dynamic leg press and leg extension 1-RM. However, the practicalities of undertaking gym based exercise whilst concomitantly reducing daily walking renders this as an elegant proof of principle study. Applying the findings of Devries *et al.* (2015) in older adults relies on employing an exercise regime that ideally could be undertaken in real world scenarios that might induce step-reduction, such as minor injury or illness. The exercise snacking regime was therefore investigated to suit these criteria.

Chapter 3 investigated the efficacy of exercise snacking in older adults who were healthy, but not currently engaging in exercise on a weekly basis. Whilst statistical significance was not reached, there were trends for improvements in leg pressing power following 28 days of exercise snacking twice a day. A 6% increase in P_{\max} was observed, along with a 5% increase in the mean power of eight repetitions at 40% 1-RM, with large effect sizes for the difference in change scores for each index of power between the ES group and control group. Literature on body weight resistance type training interventions in older adults lasting only four weeks is scarce, particularly when training is undertaken daily, as in the case of exercise snacking (Thiebaud *et al.* 2014). In an example of the effects of short duration training studies in older adults, six weeks of progressive but submaximal resistance training increased *Vastus Lateralis* mCSA by 7% and knee extensor 1-RM by 32% (Scanlon *et al.* 2014). This demonstrates that large increases in muscle function and cross sectional area are possible in older adults, even over short training periods. In another six-week resistance training study, with training three times a week, 30% improvements in sit-to-stand score in 30 seconds were achieved (Cavani *et al.* 2002). Indeed, the primary outcome of the study reported in Chapter 3 was sit-to-stand score in a minute, which improved by 31%, alongside the more subtle improvements in leg press performance.

When applying the exercise snacking regime to a step-reduction scenario, similarly to the findings of Devries *et al.* (2015), an improvement in dynamic leg press performance appears to have occurred. Specifically, P_{\max} increased by 5% in the SR+ES group, with a 2% increase in F_{\max} . Although these data are from a small sample size, given that undertaking no protective exercise intervention appeared to lead to reduced P_{\max} and F_{\max} , these data are still of importance. A key consideration of the work by Devries *et al.* (2015) was that the unilateral exercise intervention implemented may have prevented decline in leg strength in the non-exercised leg by a cross-education effect (Carroll *et al.* 2006). As such, direct comparisons in functional changes in the two groups in the present study, and between the exercise and non-exercised legs of participants in the study of Devries *et al.* (2015) are not straight forward. Furthermore, given the stark difference in the manner in which the exercise snacking was implemented between studies described in Chapters 3 and 4, drawing direct comparisons between the magnitudes of responses perhaps is not pertinent either. In any case, it seems that exercise snacking may be an efficacious strategy to increase leg muscle function in older adults, and mitigate function losses during periods of reduced activity.

Alongside functional changes with exercise snacking, it seems that there is also potential for the exercise snacking regime to increase muscle cross sectional area, in both free living and reduced activity periods. Chapter 3 observed a trend towards a significant increase in thigh mCSA, and a large effect size for the difference in change scores between groups, albeit the increase being only 2% in the ES group. In the participants undertaking exercise snacking three times a day during step-reduction in Chapter 4, at the lower thigh there appeared to be a 3% increase in mCSA, and 4% increase at the mid-thigh. It should be noted that in neither study implementing exercise snacking was there an increase in leg lean mass measured by DXA, despite Sherk *et al.* (2014) previously demonstrating strong correlations between pQCT and DXA for soft tissue analysis. The mechanism by which exercise snacking might induce hypertrophy could be related to the regularity of the exercise stimulus creating an increased sensitivity of muscle to aminoacidemia for proportionally more of the time (Dankel *et al.* 2016; Witard *et al.* 2009). Interestingly, in the study described in Chapter 4, there still appeared to be a moderate degree of hypertrophy despite

participants apparently not achieving what is regarded as optimum protein content in the diet, particularly during reduced activity (Phillips *et al.* 2016).

In older adults, fall risk is often associated with age related loss of strength (Butler *et al.* 2008), and evidence suggests that both strength and balance may be compromised by short term periods of inactivity (Kouzaki *et al.* 2007; Mulder *et al.* 2014). Exercise is regarded as an important component of falls prevention interventions (Rose and Hernandez 2010), although Muehlbauer *et al.* (2015) found that correlations between balance measures and leg power or strength were small. The full extent to which strength exercises contribute to balance in older adults is still not clear (Howe *et al.* 2011), and so a variable of interest in relation to the exercise snacking was postural sway, as an indicator of balance. The CoP mean speeds and AP ranges of participants in the studies of the present thesis were in line with those reported in previous research in older adults (Prieto *et al.* 1996; Melzer *et al.* 2004; Fukusaki *et al.* 2016). However, neither the exercise snacking nor the step-reduction had notable beneficial effects on postural sway in the present thesis. Moreover, CoP mean speed with feet together and eyes closed in Chapter 3, and AP range with feet together and eyes open in Chapter 4, both actually appeared to be worse compared to control or with step-reduction with no exercise snacking respectively. There is no obvious explanation for this, other than to suggest that the feet together conditions were single trials without repeats, so perhaps may have been subject to greater variability in data.

The participants described in Chapters 3 and 4 were broadly comparable in indices of postural sway (CoP mean speed and AP range) between the two studies, although postural sway when feet were positioned closed together appeared to be lower in the participants in Chapter 4. This difference may be due to participants in Chapter 4 being barefoot during testing, and participants in Chapter 3 being shod, as footwear may actually increase postural sway in older adults (Brenton-Rule *et al.* 2011). Interestingly, and for context, the actual risk of falls seems to in fact be 1.3 fold higher in bare feet or stockings compared to when wearing shoes in older adults (Koepsell *et al.* 2004). To some extent, this fact alone does undermine the use of static postural sway measures of balance for inferring fall risk, as of course risk for trips and falls typically occur during ambulation rather than during quiet standing. Indeed, measures of dynamic stability may have added insight into the possible balance related

adaptations that could occur due to an exercise snacking or step-reduction intervention. In particular, assessment of the ability to adapt to perturbations in balance may have provided a more real world indication of fall risk, given the complex and multifaceted coordination required to maintain postural equilibrium (Horak 2006). Margin of stability is a measure that progresses the concept that to maintain balance in a static position, centre of mass must remain of the base of support, to account for the velocity of the centre of mass during dynamic movement (Hof *et al.* 2005). There is convincing evidence that balance training improves margin of stability in older adults during ambulation and balance perturbation exercises (Arampatzis *et al.* 2011). However, it is not clear whether resistance type exercise training and improvements in strength improve margin of stability beyond balance training alone in older adults (Bierbaum *et al.* 2013). In any case, whilst perturbation of balance measures were considered for inclusion in the studies of Chapter 3 and 4, with the range of measures implemented this would have been logistically challenging.

5.3.3 Mechanistic impact of step-reduction

Chapters 3 and 4 included measures of neural drive of the plantar flexors to characterise a potential mechanism by which force or power may be increased or decreased by exercise snacking or step-reduction. In simplistic terms, literature suggests that whilst total number of available motor units represents a muscles potential to generate force, the ability to ‘switch on’ those motor units may be the regulator of force production (Belanger and McComas 1981). It is suggested that neural drive can be considered as a ratio of motor units recruited to total motor units, estimated from the force of maximal voluntary contractions compared to the force of interpolated twitch stimulations during maximal voluntary contractions (Behm *et al.* 2001). Neural drive appears to be a reasonably plastic variable, responding to loading and unloading in the space of weeks (Hvid *et al.* 2016; Gondin *et al.* 2004). However, in Chapter 3, there was no change in interpolated twitch ratio, and indeed there was a notable heterogeneity in the data from both the ES group and the control group. In Chapter 4, it was not possible to assess interpolated twitch ratio at all in the SR group, however in the SR+ES group there was the same distinctive variability in the data at all times points measured. Folland and Williams (2007b) and Shield and Zhou (2004) describe at length the methodological challenges associated with employing the twitch interpolation technique, and it was considered that two familiarisation sessions with maximal isometric plantar flexion and twitch interpolation would have been sufficient to obtain consistency in data. Given that the SR+ES group had undertaken the test on five occasions, albeit either side of interventions, and the data were still highly variable, it is likely that methodological errors were affecting the data.

5.4 Future Directions

The physiological and biomechanical impact of severe forms of muscle unloading on both muscle and whole body function and anthropometrics has received much research attention, both in younger and older adults (Trappe 2009). However, given that bed rest occurs very rarely in generally healthy adults, how short-term reductions in physical activity impact muscle function and size is perhaps more relevant to the general population. As such, the bulk of the present section will be focussed specifically around research questions which build on the findings of chapters 3 and 4, on exercise as a means to attenuate the negative consequences of reduced activity, and further questions surrounding step-reduction itself, in an aged population.

Firstly, it is crucial to emphasise that the studies of the present thesis only considered whole limb function *in vivo*, and as such, it is impossible to speculate on whether the adaptations seen in strength or power were down to changes in the muscle tissues mechanical properties themselves. Whilst this may be a somewhat academic point, with real world function *in vivo* being the important aspect for older adults, understanding the mechanisms by which the changes in function take place is an important progression in the scientific study of this area. Future research to understand the plasticity of muscle tissue itself in response to reduced physical activity or exercise snacking through single fibre contractile property work would help to refine interventions and treatments for sarcopenia in the future. Indeed, this would allow for further the comparison to other literature in the field. Similarly, there are potentially more subtle measures of whole body function *in vivo* that could be employed to study the potential impact of both exercise snacking and step-reduction models. For example, the collection of kinematic and kinetic data whilst performing maximal sit-to-stand or ambulatory tasks could be used to calculate centre of mass mechanical work or power. Deeper analysis of movement patterns may also help to understand improvements in functional tests employed in the present study, for example, gait analysis indices such as energy recover or duty factor during walking could explain how improvements in 6MWT distance occurred without a perceived increase in exertion in the ES group of Chapter 3.

The two previous studies exploring the step-reduction model of disuse in older adults, and the present findings, leave many unanswered questions regarding the full extent to which old muscle responds to short term reductions in physical activity. Whilst findings from Breen *et al.* (2013), Devries *et al.* (2015), and the present findings suggest that isometric strength does not appear to be affected by step-reduction, the mechanism by which dynamic power and strength appeared to be lost in the present study requires further investigation. Bed rest has been shown to reduce neural drive (Kawakami *et al.* 2001), however despite attempts to address this by assessment of plantar flexion twitch interpolation, the present thesis does not meaningfully contribute. Further research should consider a more thorough assessment of neural changes in response to step-reduction, particularly in the knee extensors where differences appear to exist between dynamic and isometric muscle contractions.

The time course of changes in muscle function due to step-reduction may also be a consideration in future research. Whilst the present thesis did not observe obvious decreases in muscle size as was observed by Breen *et al.* (2013), Wall *et al.* (2014b) has demonstrated that muscle mass loss may be accelerated in the early stages of muscle disuse. Whether muscle function changes occur in a corresponding time course may be of interest. If the change in function is more gradual then protective exercise may not be so important in the early days of step-reduction, which may be relevant to injury or illness recovery. Moreover, there is currently no understanding on the role prior physical activity levels play on the response to the step-reduction. It may be that higher baseline activity levels actually exaggerate step-reduction induced function loss, due to the greater relative change in activity levels. Equally, there may be a protective effect of high activity levels before step-reduction. In instances in which a period of reduced activity is scheduled, for example with elective surgery, then a short term increase in activity could be undertaken prior to reducing physical activity. Whether increased baseline physical activity is protective against muscle loss or not, it may be that undertaking a period of resistance exercise before a period of inactivity could be implemented to increase muscle mass and function, such that any loss induced by the reduced activity would be negated compared to pre-exercise levels.

The findings of Chapter 3 and 4, again with very limited data, suggested that postural sway was neither improved with exercise snacking, nor particularly affected

by step-reduction. Whilst postural sway measures are relatively easy to conduct, older adults are at a limited risk of falling when standing quietly. For over 20 years it has been recognised that risk of falls the older adults is high immediately following bed rest (Bloomfield 1997), and is increased in the month following hospital stays as short as two days even (Mahoney *et al.* 1994). Given that the findings of Chapter 4 demonstrate muscle power and force may be decreased with step-reduction, and exercise snacking may alleviate this negative outcome, whether gait related changes, and in particular trip recovery mechanics are altered warrants further investigation. Equally, whether dynamic dual-task tests that assess balance during ambulation would be effected by exercise snacking or step-reduction may also be an interesting question with perhaps more real-world applicability than static balance tests.

In both scenarios in which the exercise snacking regime was employed, in free-living (Chapter 3) and during step-reduction (Chapter 4), the adherence was very high. It should be noted that all participants volunteered for the studies included in this thesis, thus it would be expected that adherence behaviours might be high (McCambridge *et al.* 2014). Certainly, adherence to the exercise snacking in other groups would require further consideration, as the characteristics of the exercise snacking programme oppose those highlighted by Thiebaud *et al.* (2014) as effective for increasing adherence insofar it was not supervised. Moreover, the actual exercise selection, intensity, and how often it is recommended to undertake exercise snacks all have scope for optimisation (Dankel *et al.* 2016). Indeed, a mechanistic understanding of how the exercise snacking might lead to improvements in leg press power is lacking. Neural adaptation could play a role, however the methodology used in Chapter 3 and 4 would need to be refined to investigate this further. Equally, in a large enough sample size and carefully designed study, whether an increase in mCSA contributed to increased power could be investigated (Dankel *et al.* 2017). As a subsequent application to follow on from the findings of the present thesis, it would be interesting to explore the use the exercise snacking model in older adults undertaking non-volitional reduced activity, for example during hospital stays. Furthermore, investigating the potential for an exercise snacking model to improve functional capacity in already functionally compromised older adults, for example those in assisted living settings.

Finally, as may be evident from the limited sample size, the study reported in Chapters 4 exploring exercise snacking and step-reduction remains in a largely unfinished state. Due to the limited number of participants completing the interventions, there were a number of mechanistic biochemistry measures that have not been analysed for the present thesis. Chiefly, the study originally set out to determine integrated rate of MPS using deuterated heavy water as an orally ingested tracer, as has subsequently been undertaken by McGlory *et al.* (2017). Two muscle biopsies were taken on the pre- and post-intervention trial days, with additional biopsies taken three days and eight days into the step-reduction period, and three days after the step-reduction period had finished. The aim of these sample timings was to allow inspection of the potential change in rate of MPS over the course of the step-reduction, and initial three days of returning to habitual activity levels. Another line of investigation associated to the muscle biopsies was to be an investigation into the ‘stress response’ to resistance exercise before and after the two-week step-reduction period. Analysis of markers of both systemic inflammation and circulatory heat shock protein concentrations, along with intramuscular heat shock protein response, were to be conducted to further understanding of the impact of reducing physical activity on the stress response to exercise. Alongside this, the amplex red live tissue assay to examine the role of mitochondria as a source of oxidative stress in a resting state before and after step-reduction was attempted. Due to technical difficulties or insufficient yield from muscle biopsies, this analysis was abandoned.

5.5 Conclusion

This thesis has highlighted that in healthy, active, older adults, habitual physical activity *per se* does not predict maximal power, strength, or speed, during maximal effort leg pressing. However, measurable increases in power and strength can be achieved in healthy, reasonably active but non-exercising older adults in as little as one month by undertaking five minute bouts of homebased exercise twice a day. When two weeks of severe physical activity restriction by limiting daily walking to <1,500 steps was undertaken without exercise snacking, older adults were shown to potentially lose leg pressing force and power, despite no measureable decrease in muscle size. However, when performed three times a day, the exercise snacking regime was demonstrated to potentially increase leg press power and thigh muscle cross sectional area in older men even during a period of very reduced activity. In summation, the exercise snacking regime may be a promising model for maintaining or increasing muscle mass and strength in older adults, and could be also be considered as a means to protect against loss of muscle function during periods of reduced physical activity.

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